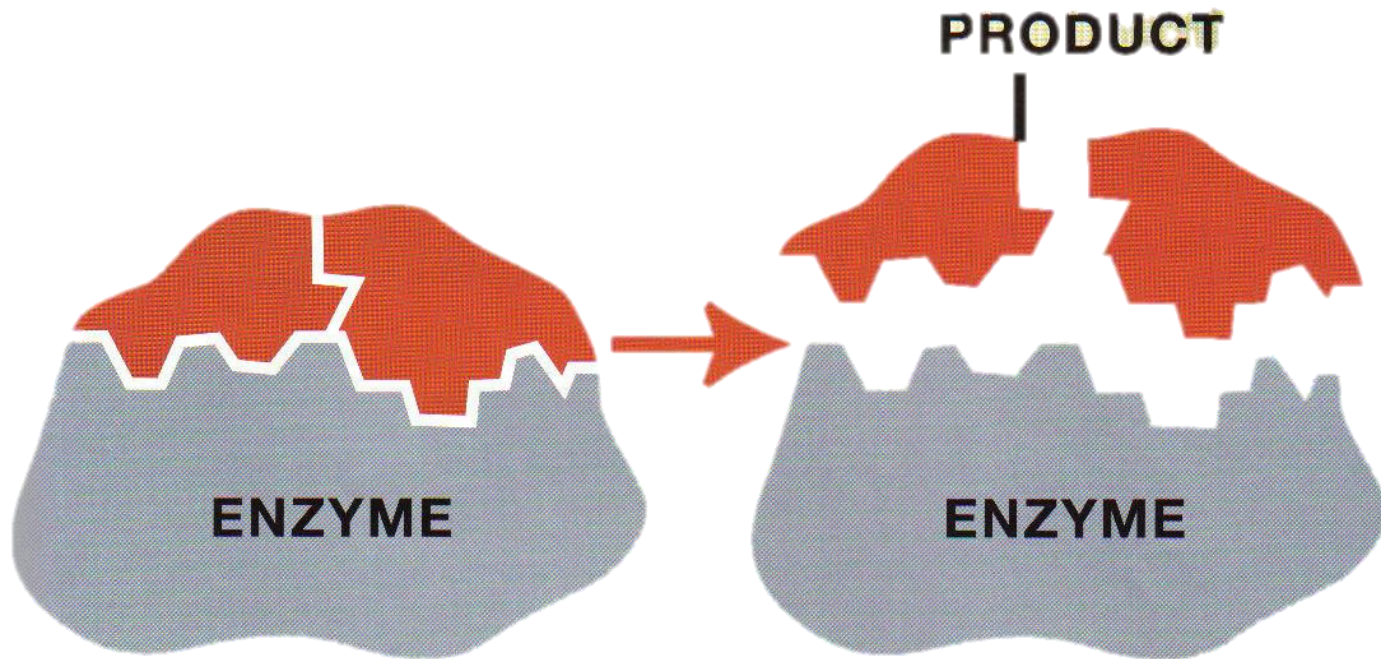




Cambridge Assessment
International Education

AS Level
Chapter 3

Enzymes



Chapter Outline

Part 1: Enzyme Structure and Mode of Action

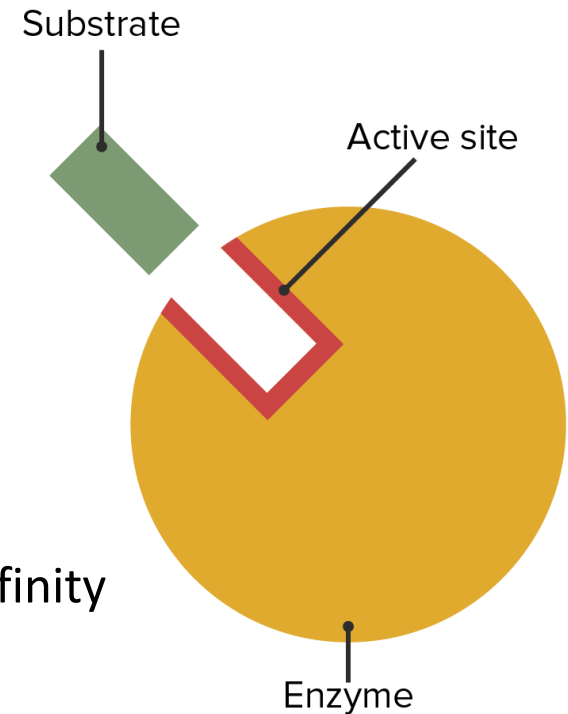
- Enzyme Structure
- Lock and Key Hypothesis vs Induced Fit hypothesis
- Mode of Action

Part 2: Mechanism of Enzymatic Reaction

- Measuring the rate of reactions
- Time course graph

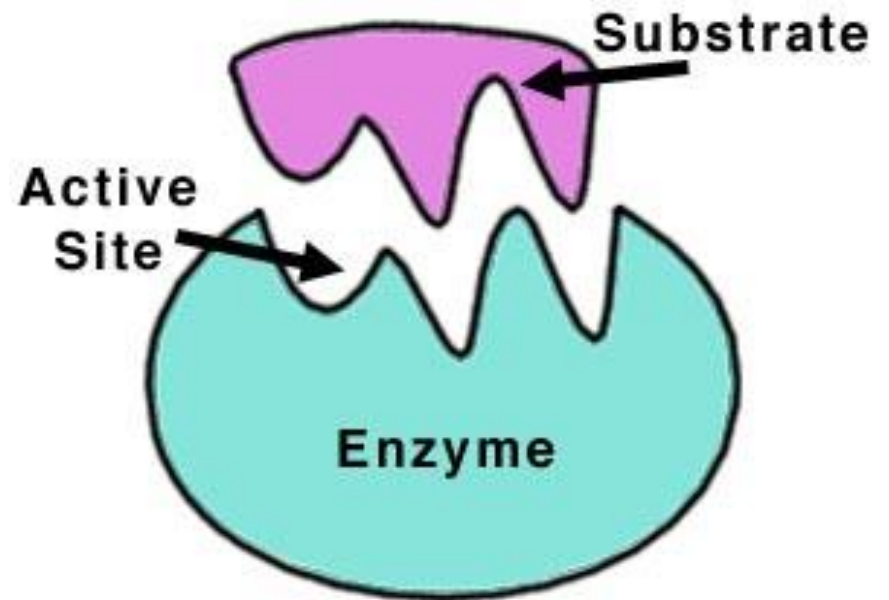
Part 3: Factors Affecting Enzymatic Reaction

- Substrate concentration
 - Maximum rate of reaction ($V_{\max}$)
 - Michaelis-Menten constant (K_m) – a measure of affinity
- Enzyme concentration
- Temperature
- pH
- Competitive vs Non-competitive Inhibition



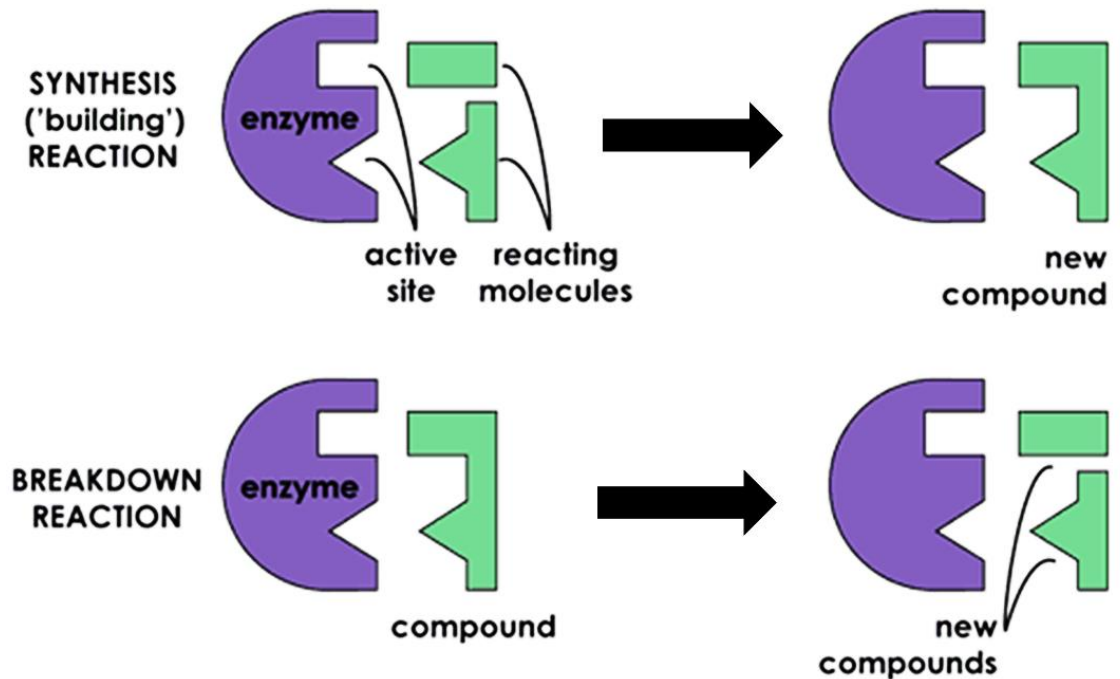
Part 4: Immobilised enzymes

Enzyme Structure



What are enzymes?

- Biological **catalysts**
- Can break down or synthesize products
- Used to control metabolic reactions

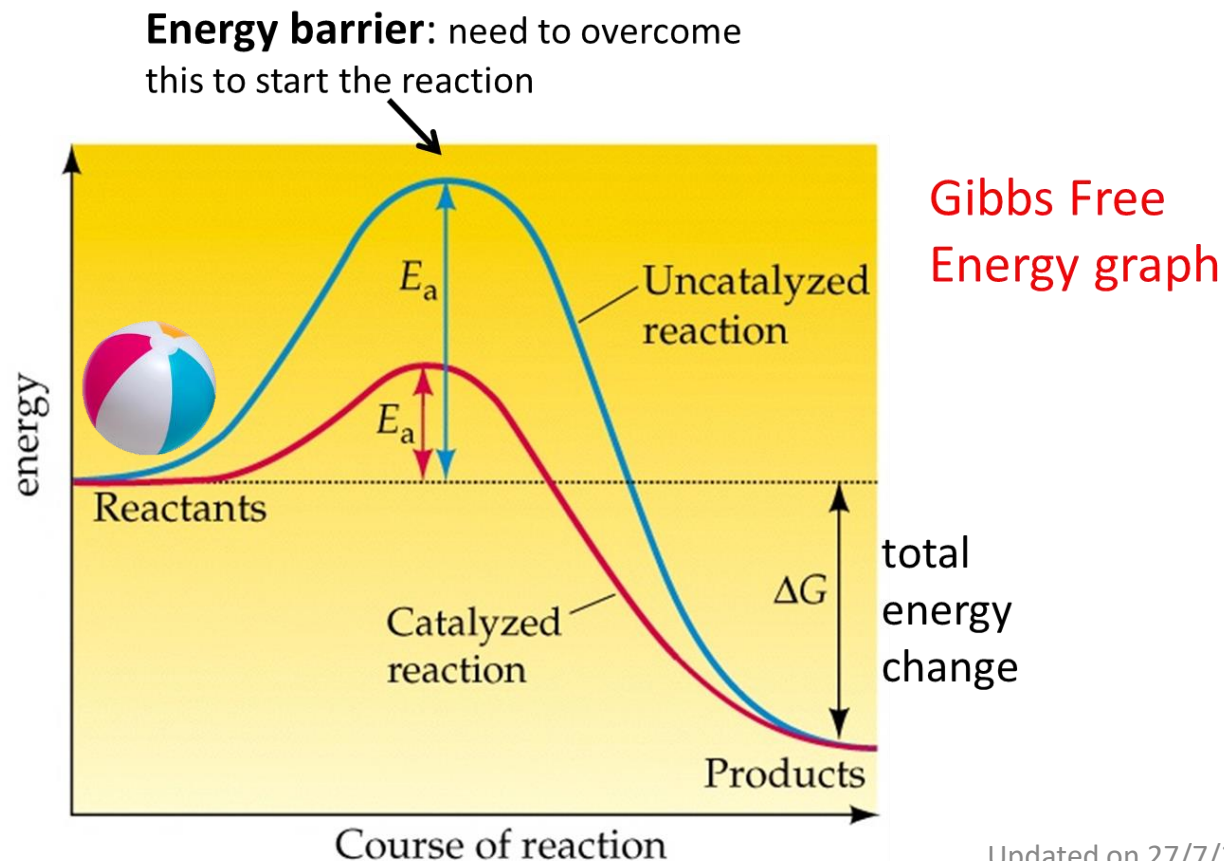


Features:

- **Speeds up / increase rate** of chemical reaction
- **Specific**: 1 enzyme to only one / few substrates
- **Unchanged** at the end of the reaction
- Effective in small amounts
- High turnover number = can catalyse many reactions per unit time

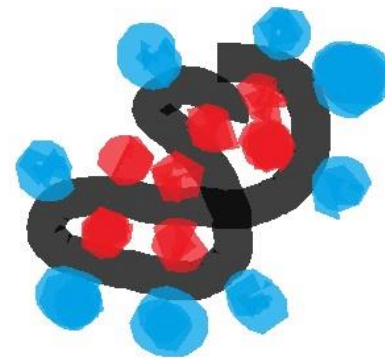
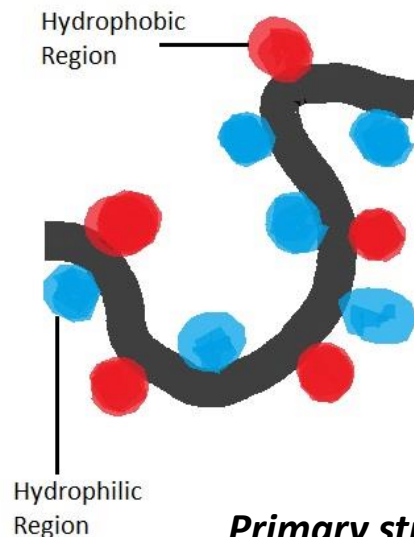
How Do Enzymes Catalyze Reactions?

- Enzymes **lower the activation energy** of chemical reactions
- Activation energy (E_a) = Energy needed for a chemical reaction to *successfully* form products
- This **provides an alternative pathway** for reactions to occur



All enzymes are globular proteins

- **Spherical** / ball shape
- Mostly **tertiary** structures, some quaternary
- Have precise **3D** structure due to interactions between R groups
- **Soluble** because...
 - **Hydrophobic R groups** are **inside**
 - **Hydrophilic R groups** faces **outside**

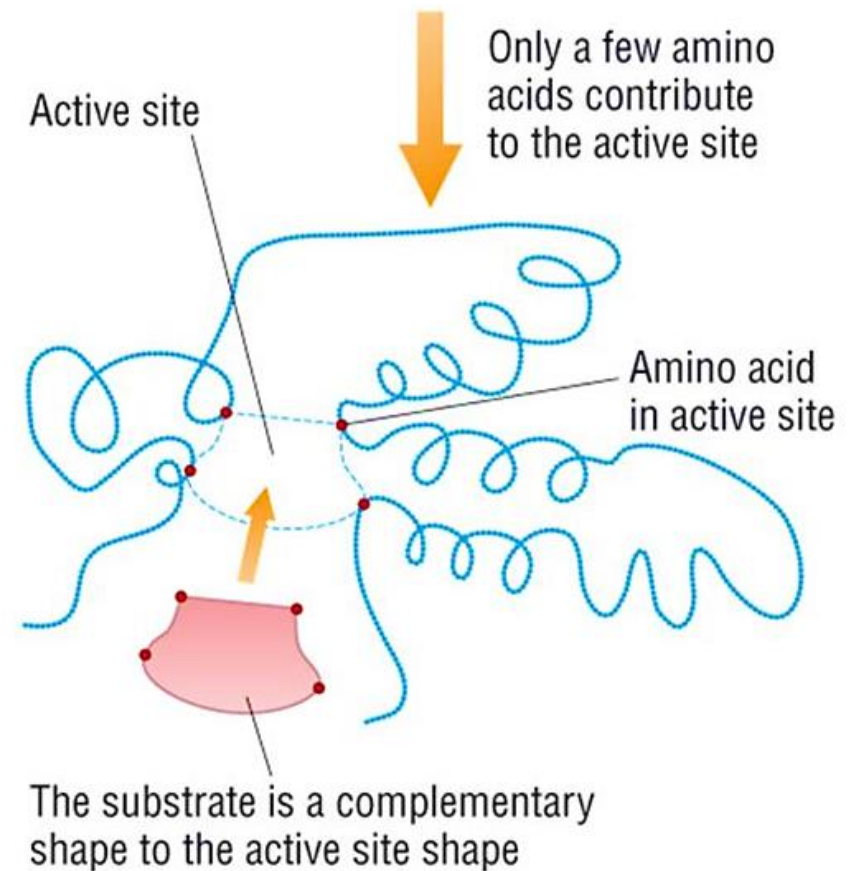


Primary structure → Tertiary structure

Active Site of Enzymes

All enzymes have an **active site**

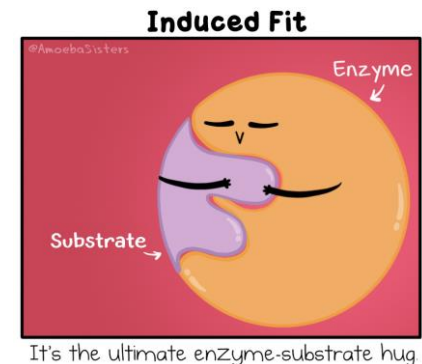
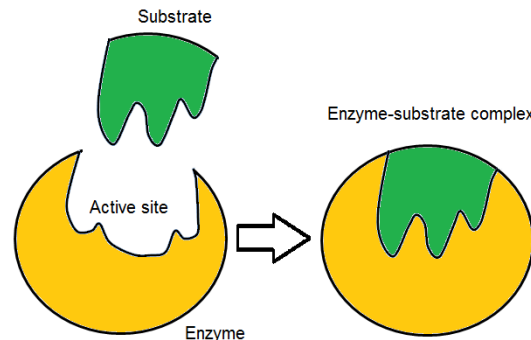
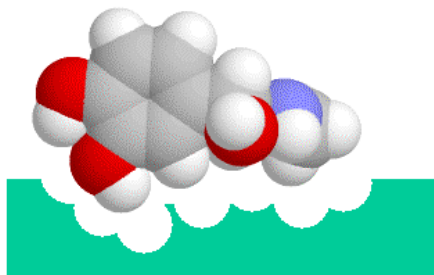
- A site where **substrates bind** to
 - Bind = **complementary** in shape to substrate
 - Active site gives **specificity**
 - Only ***few*** catalytic amino acids at the active site!
- Precisely positioned
- Not necessarily beside each other in primary seq



How do substrates bind to enzymes?

More recent!

Lock and Key Mechanism	Induced Fit Mechanism
Active site does not change shape	Active site is flexible and moulds around substrate
Shape of active site is fully complementary to shape of substrate	Shape of active site is partially complementary to shape of substrate
Result: Substrate expected to fit exactly!	Result: Better fit!



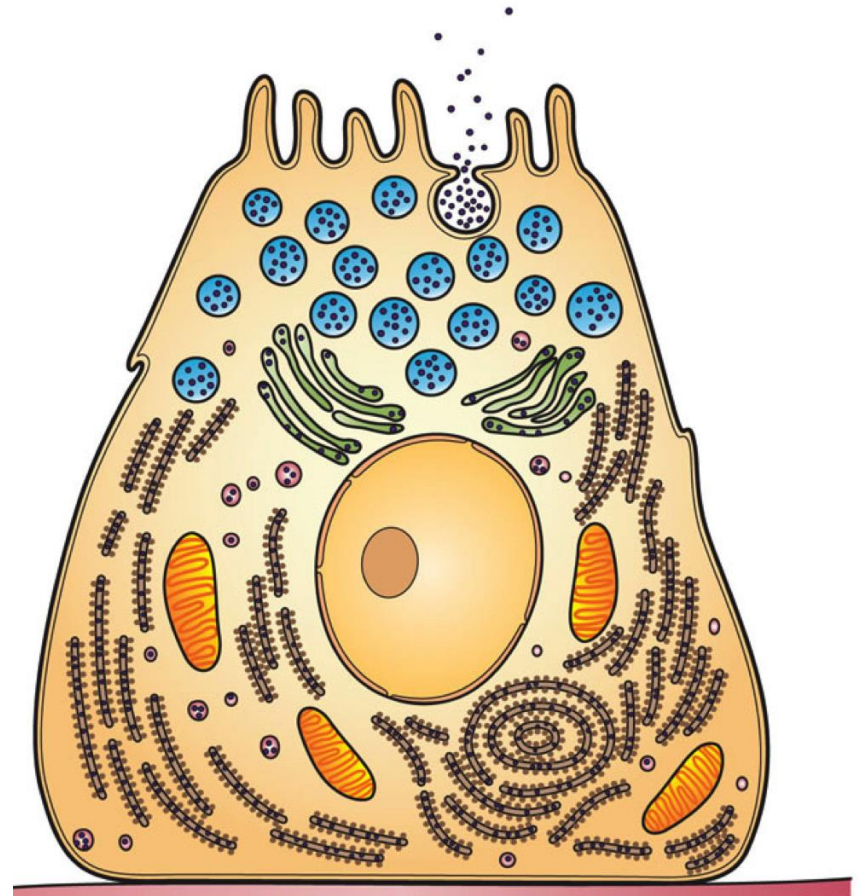
Where do enzymes operate?

Some intracellular (inside cells)

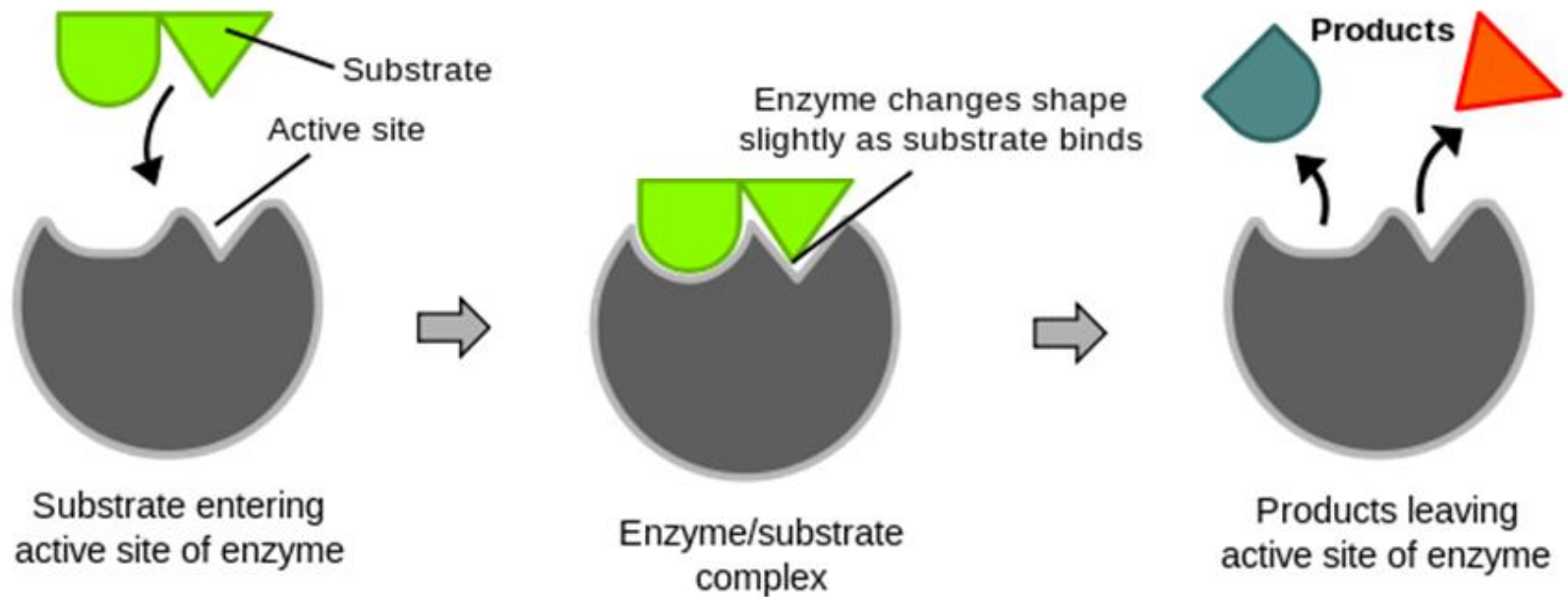
- Cells synthesise enzymes and retains them for internal use

Some extracellular (outside cells)

- Cells synthesise enzymes and secrete them for external use



Mode of Action

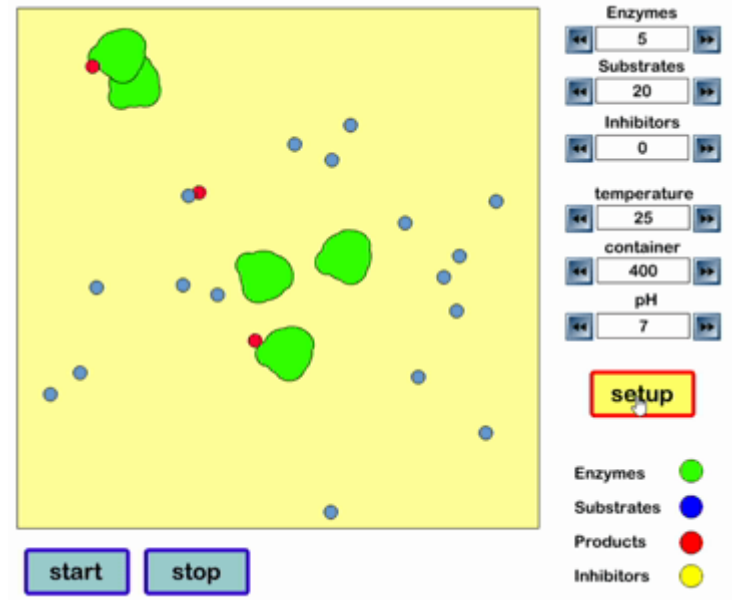


Mode of Action



1. Enzymes and substrates move and collide randomly

- Only collisions in the right orientation with enough energy result in a successful reaction
- E has **active site** with **specific** shape that is **complementary to substrate**

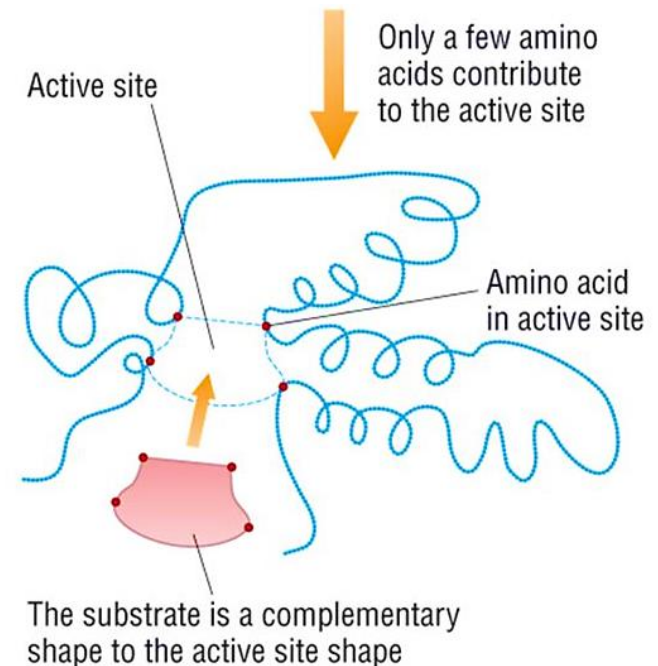


Mode of Action

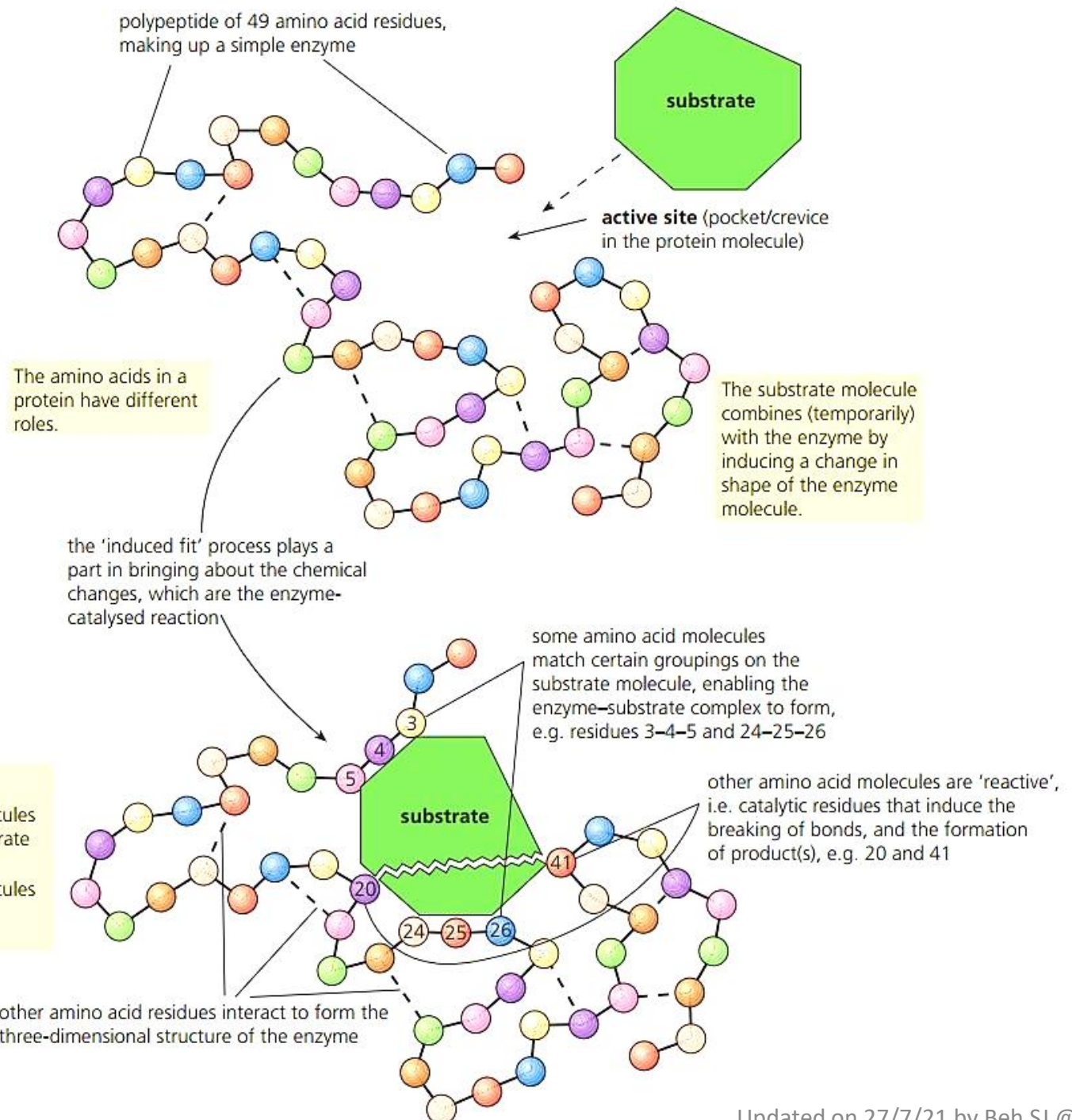


2. Formation of the enzyme-substrate complex (ESC)

- **Substrates interacts with R groups** of catalytic amino acids at the active site
 - Forms temporary bonds
 - Active site changes shape to mould around substrates (**induced fit model**)
 - **Substrates binds strongly to active site**
 - **ESC** is formed



■ **Figure 2.35**
Enzyme specificity
and the active site

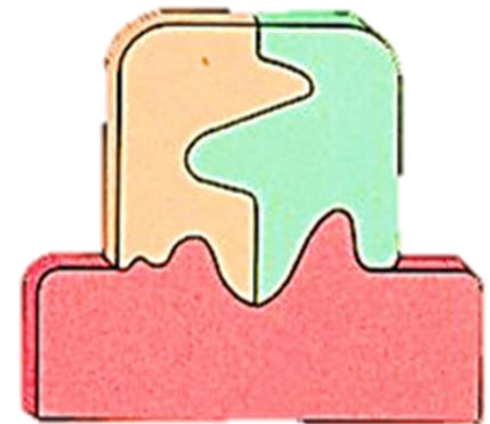


Mode of Action



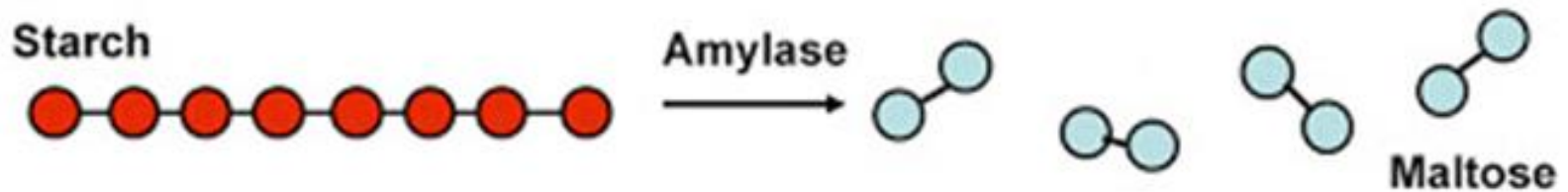
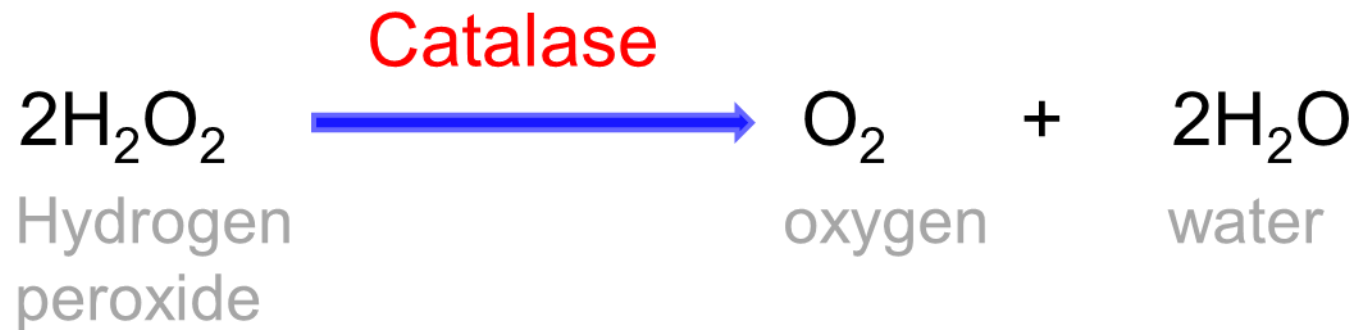
3. Product formation

- Interactions of substrates with the active site...
 - Brings substrates **close** together in the right position
 - **Put strain** on the reactants
 - So **bonds to break or form more easily**
 - Allow transfer of charges/groups
 - **Lowers activation energy**
- Products form and leave active site
- **Enzymes remain unchanged**



Interaction of S and E active site facilitates breaking and formation of bonds

Mechanism of Enzymatic Reaction

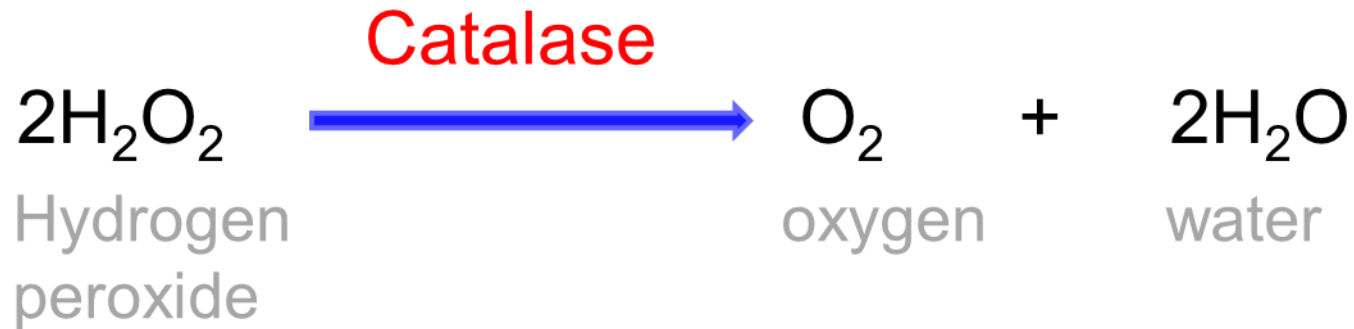


Rate of Reaction (ROR)

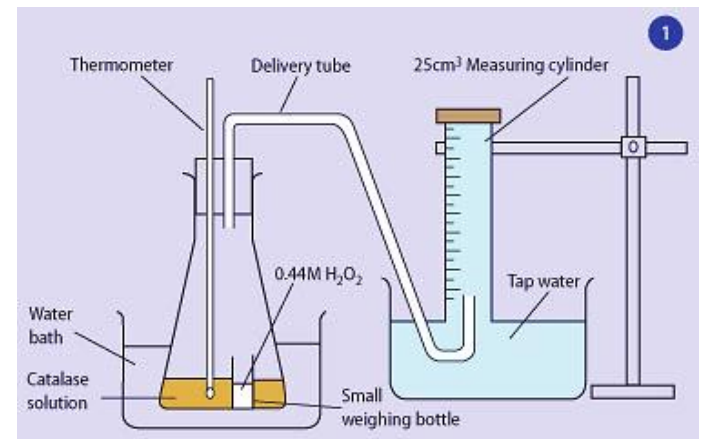
- Speed of conversion of substrate into product
 - Rate is always per unit time
- E.g. s^{-1} , min^{-1}
- How to measure ROR in an experiment? By measuring the...
 - Rate of **product formation** or
 - Rate of **substrate disappearance**
 - Steps:
 1. Measure the product formation or substrate disappearance
 2. Plot a time course graph
 3. Find the gradient of the curve = ROR

Catalase

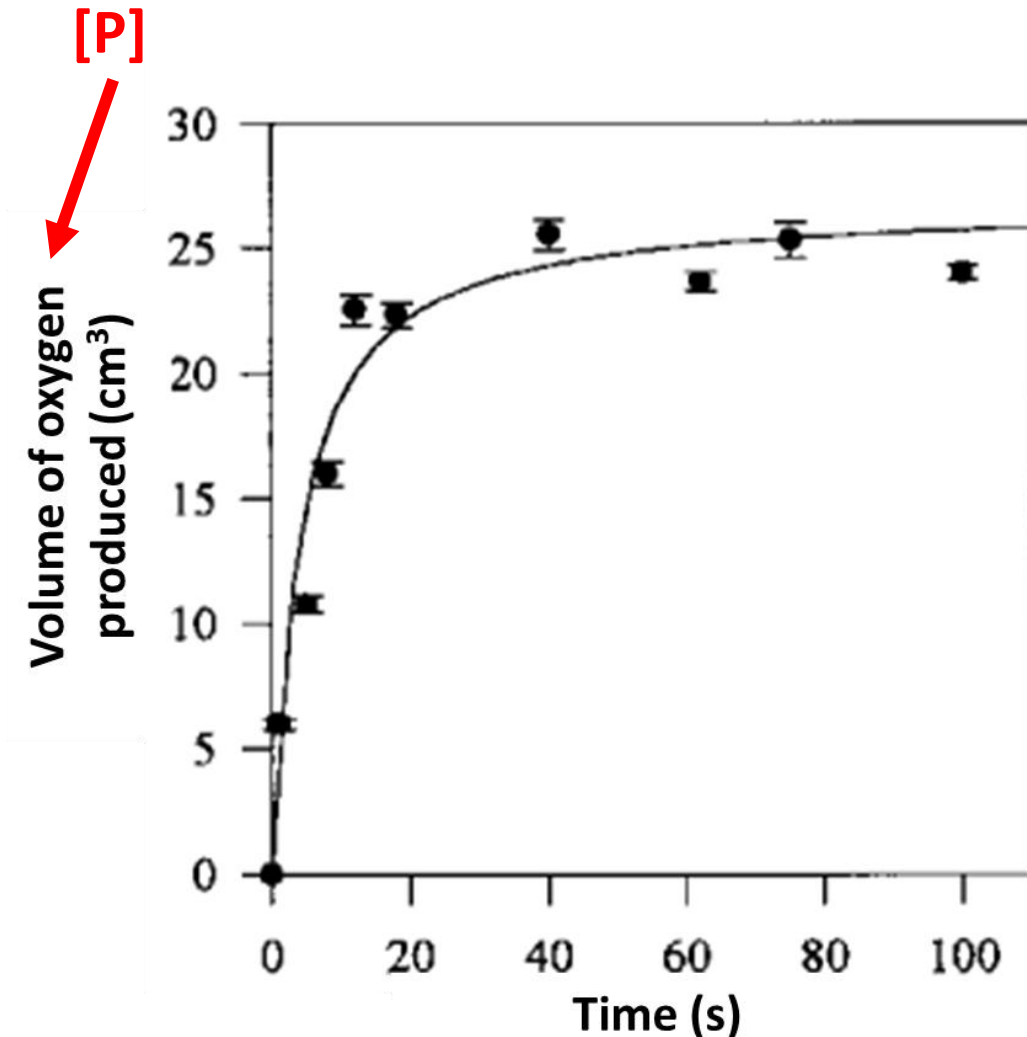
- Hydrogen peroxide is a toxic metabolic product in tissues
- Catalase enzymes are found in tissues of most living organisms



- We usually obtain it from potato / liver / yeast extract
- ROR = **Rate of oxygen released**
- **Collect gas** or count bubbles



Curve of Oxygen Produced Against Time



Volume of oxygen produced:
[P] increases then levels off

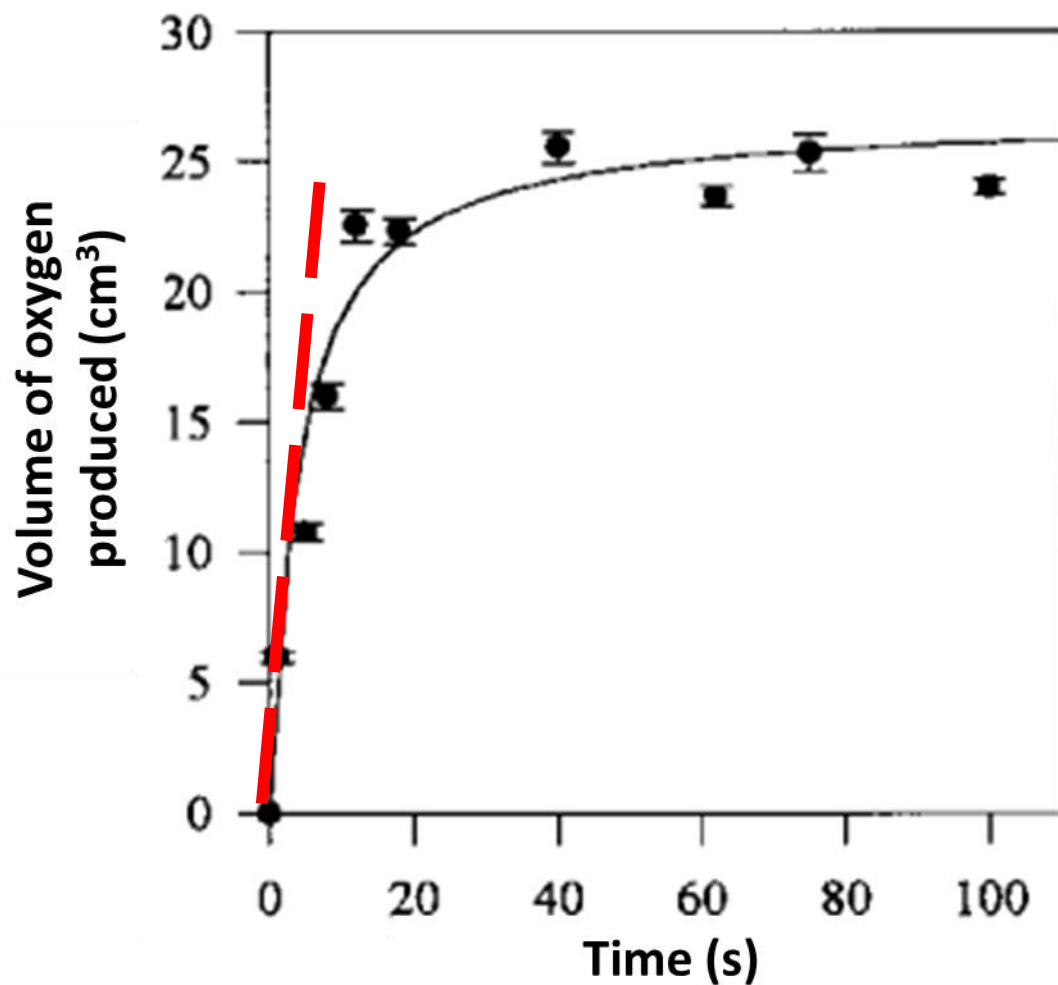
Speed of oxygen released:
Fast $\rightarrow$ slows down $\rightarrow$ stops

Rate of oxygen released:
High $\rightarrow$ decrease $\rightarrow$ 0

Rate = gradient

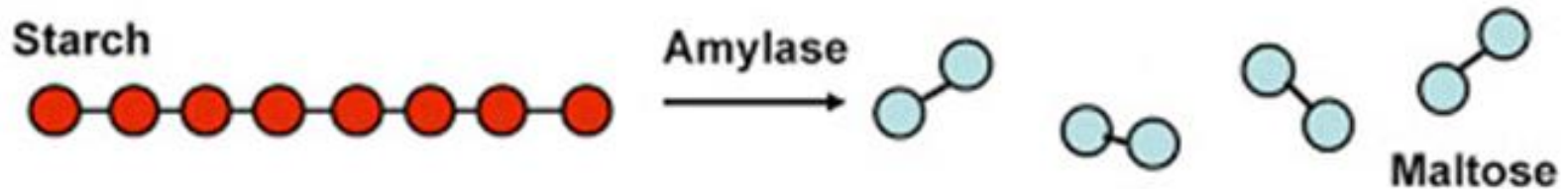
Gradient of a horizontal line = 0

Curve of Oxygen Produced Against Time



**Initial rate of reaction
= gradient of the curve at 0s**

Amylase

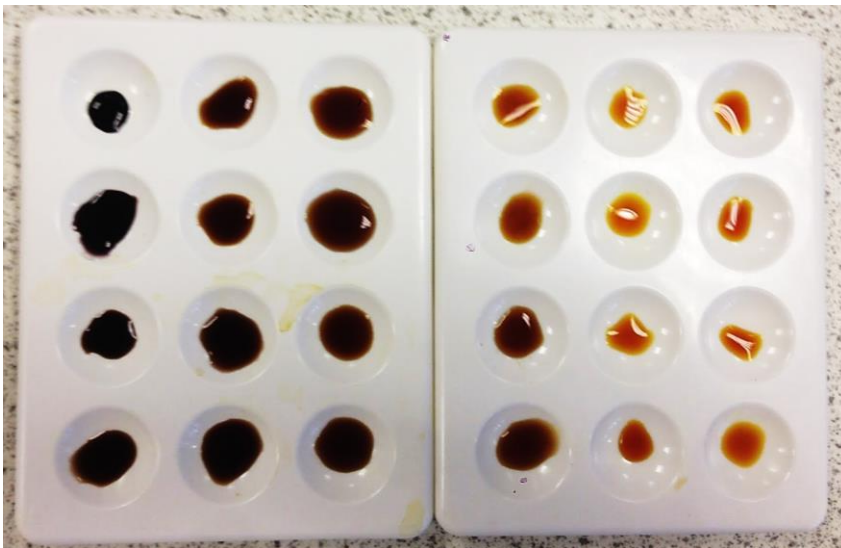


- Problem: Both substrate and products are colourless and are both liquid!
- Answer: Use iodine test!
- ROR = **Rate that starch disappears**



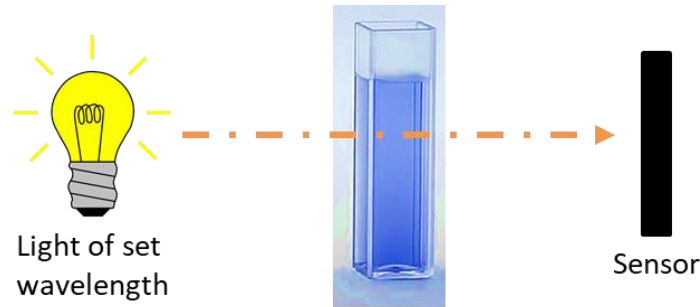
Amylase

- Test samples with iodine solution at known time intervals
- Colour will change from **dark blue** → **yellow/brown**
Dark blue: Starch still present
Yellow/brown: Starch completely hydrolyzed
- Use spotting tile... or use a **colorimeter** to measure the colour intensity



Amylase

- Use **colorimeter** measures light **absorbance** in arbitrary units (a.u.)
- Measures relative absorbance to a control (distilled water)



Distilled water
As a control
Absorbance
set as 0 a.u.

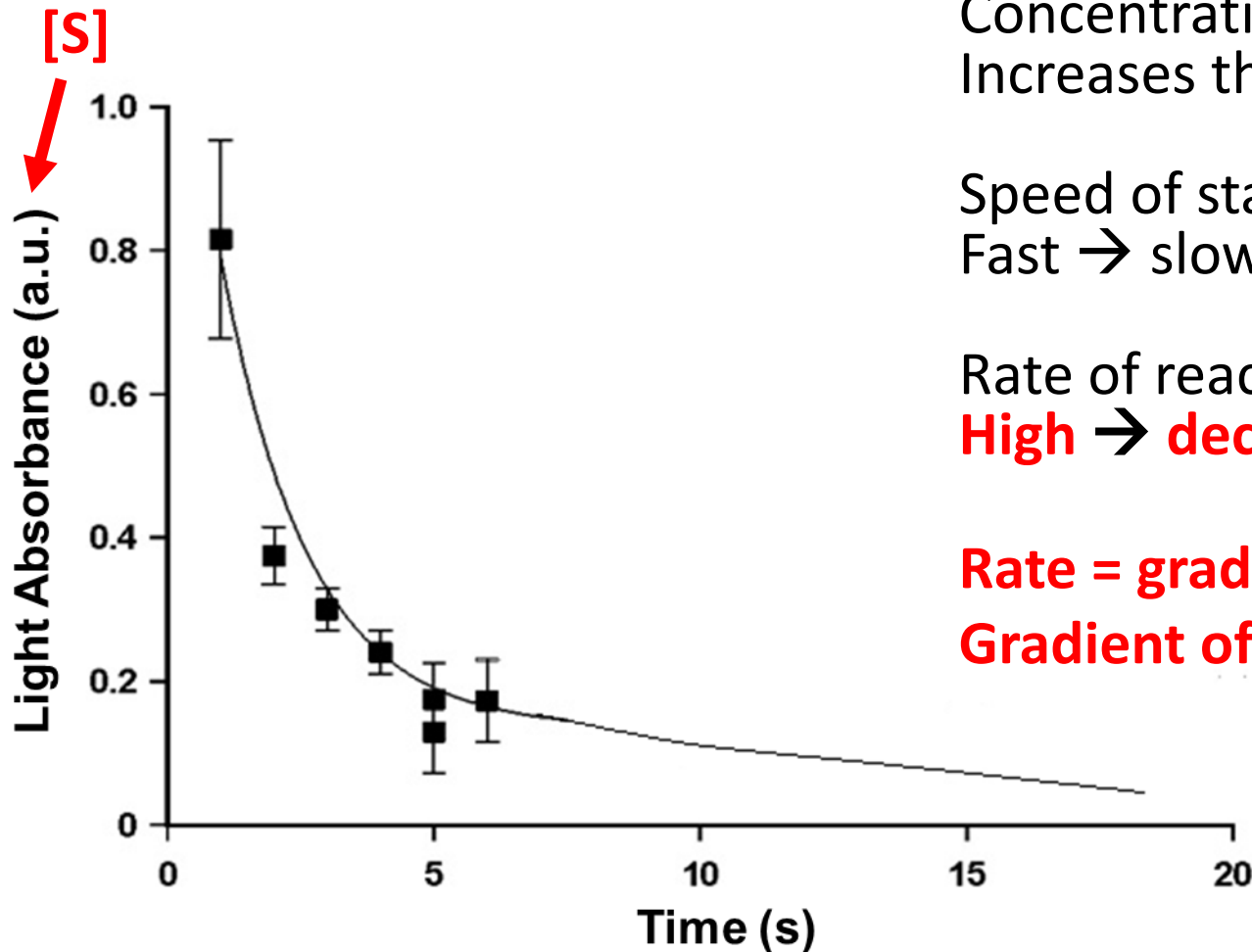


Dark blue
A lot of starch present
Absorbance is high
(relative to water)



Light yellow
No starch present
Absorbance is low
(relative to water)

Curve of Starch Remaining Against Time



Concentration of starch, [S]:
Increases then levels off

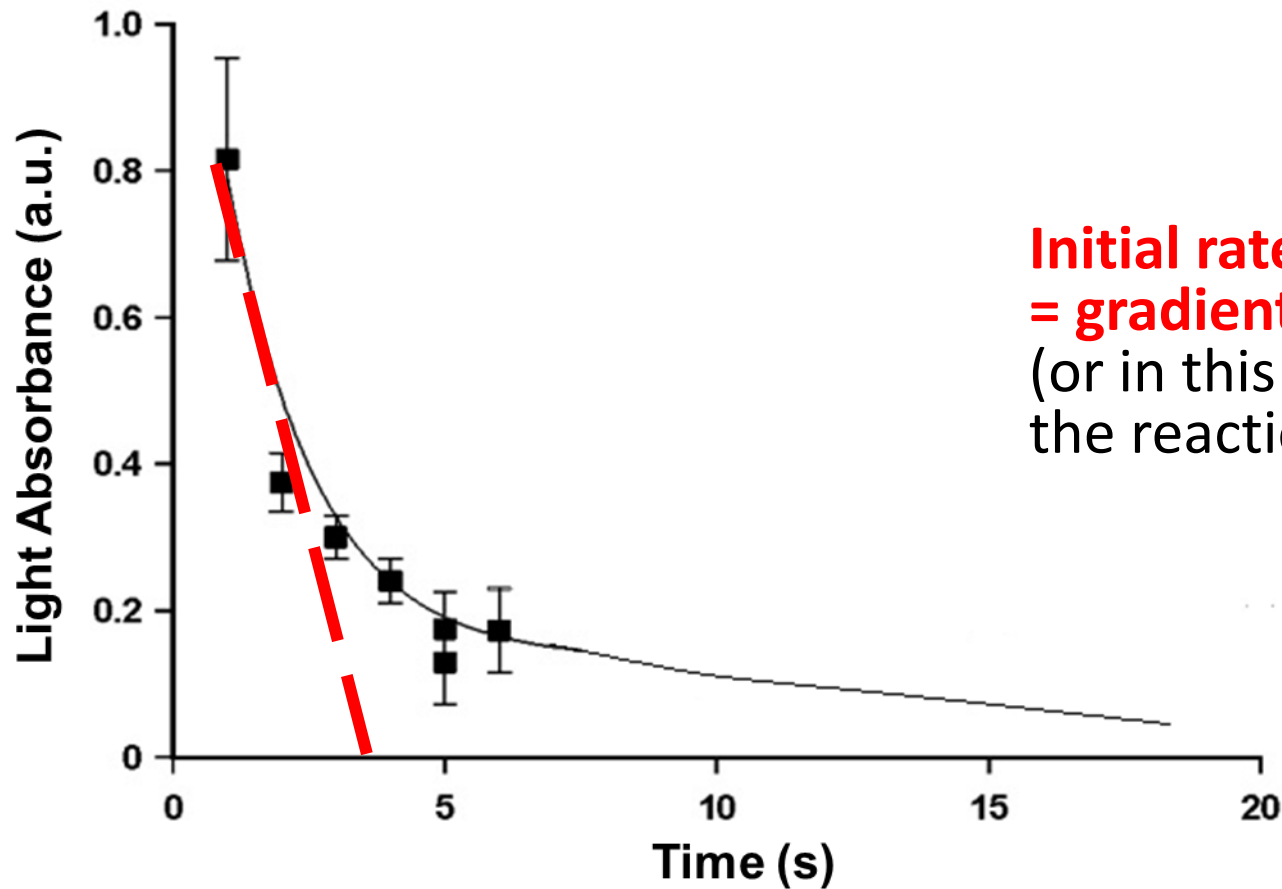
Speed of starch being used:
Fast $\rightarrow$ slows down $\rightarrow$ stops

Rate of reaction:
High $\rightarrow$ decrease $\rightarrow$ 0

Rate = gradient

Gradient of a horizontal line = 0

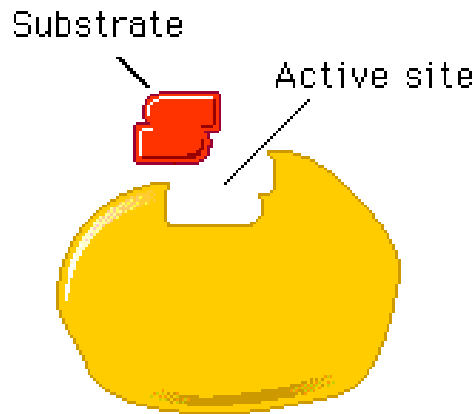
Curve of Starch Remaining Against Time



**Initial rate of reaction
= gradient of the curve at 0s**
(or in this case, as early in
the reaction as possible)

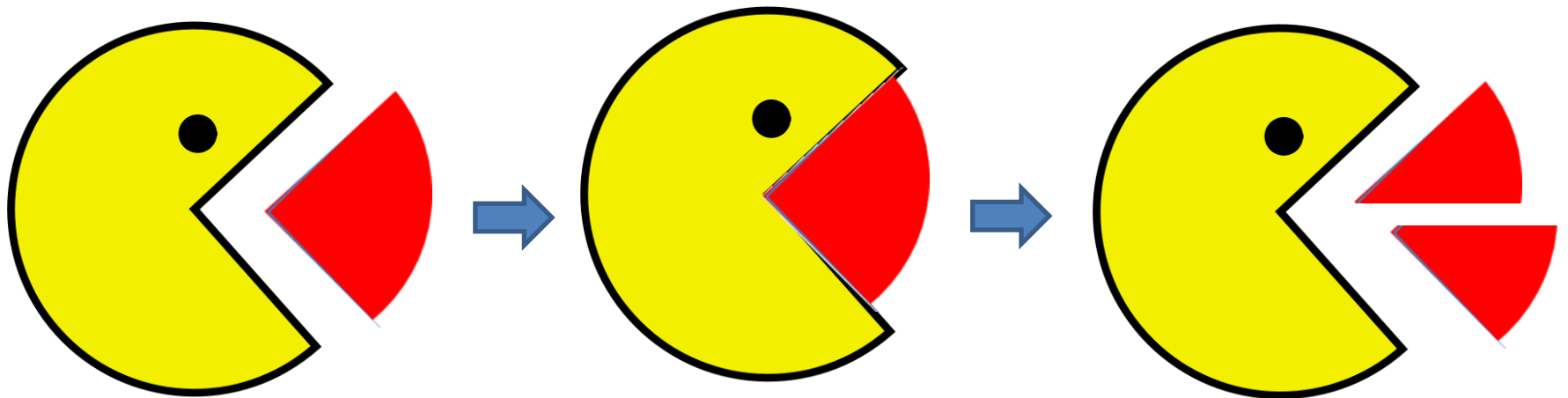
What happens during the course of the reaction?

To illustrate my next slide...



Schematic model
of an enzyme

Pretty much the
same thing, right?



The Course of Reaction

Why was the rate high at first, then decreased and became 0?

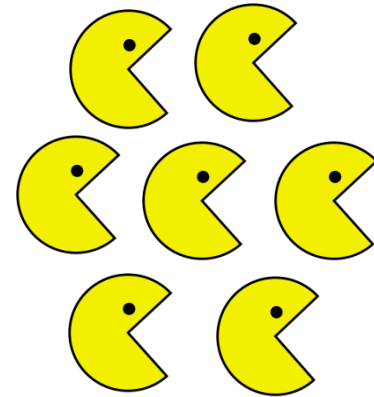
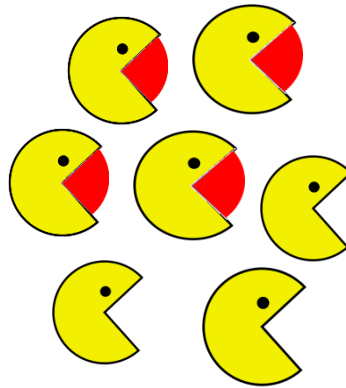
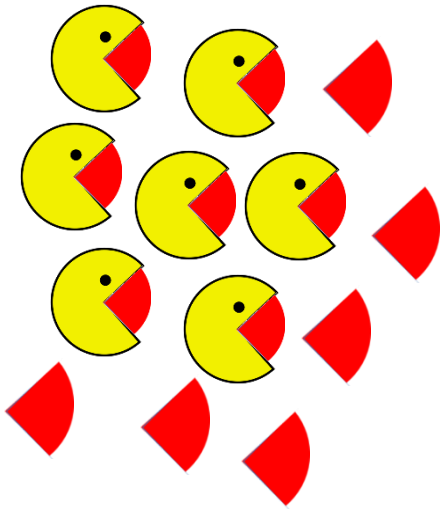
High [S]
S is in excess



Low [S]
Lesser S available



[S] = 0
No S available for binding

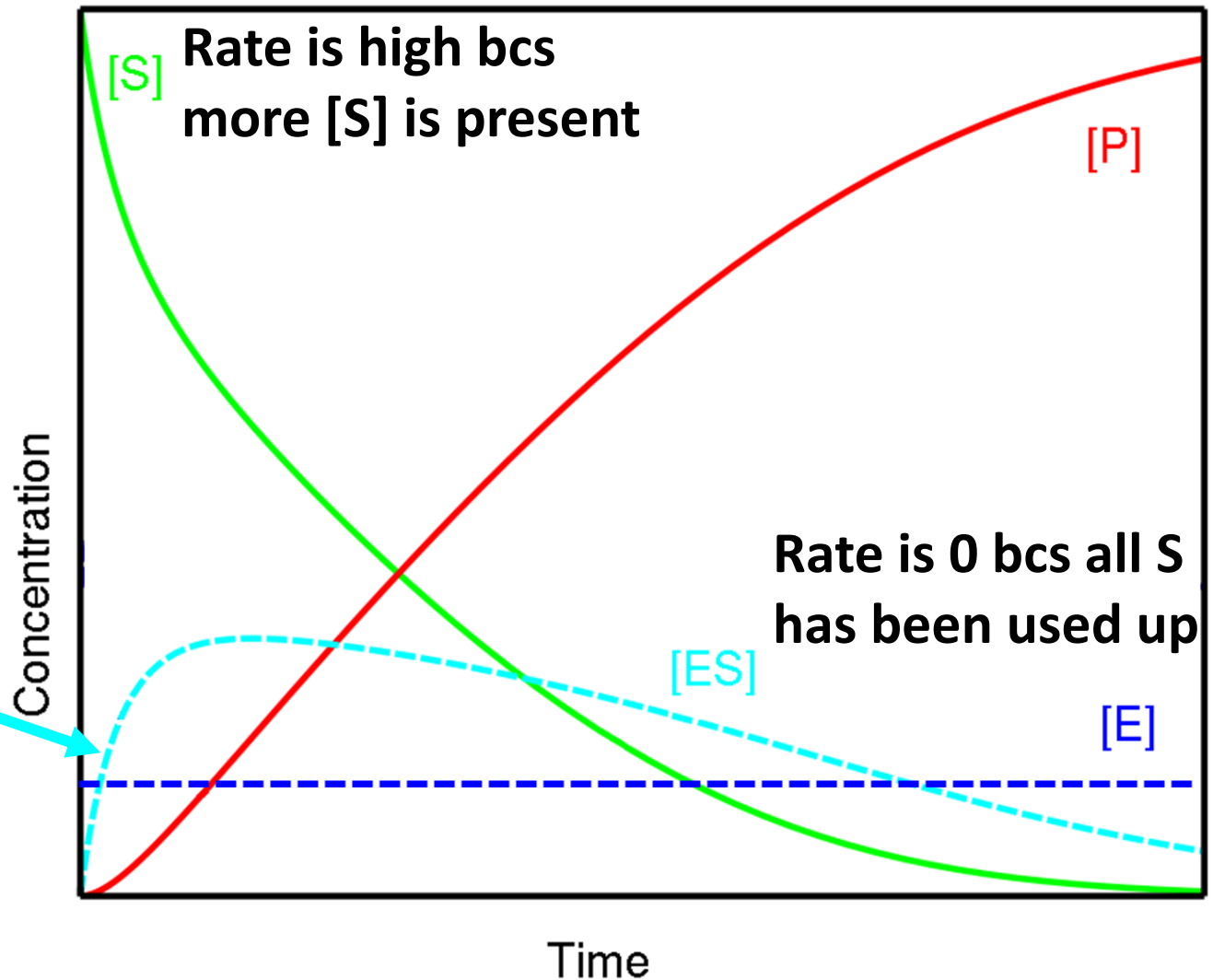


Observe that:

- P is not shown here but [P] increases over time, then levels off
- [E] = constant
- [S] reduces over time, then levels off
- **[S] the limiting factor** in this reaction

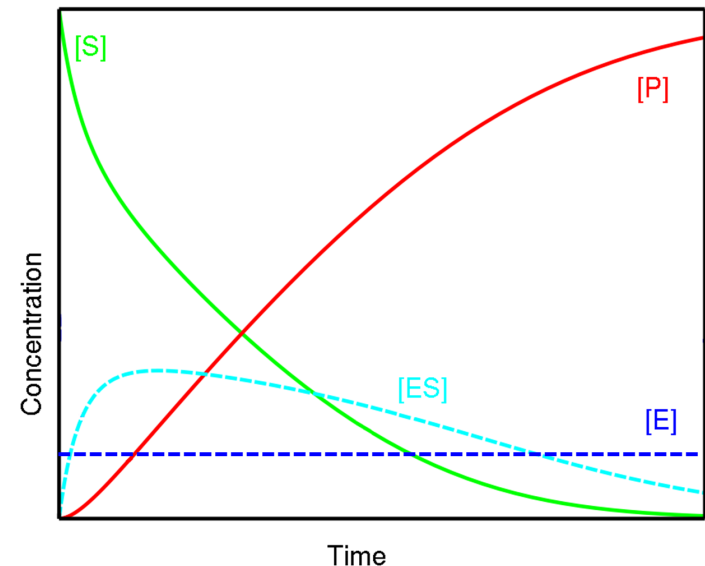
Time Course Graphs

Note that [ES] immediately increases from 0 when the reaction starts!



How to understand graphs?

- Always read the question carefully!
 - **Describe** = state your observations
 - **Explain** = explain why the observations are as such
 - **Compare / contrast** = tell me the similarities or differences between 2 sets of data
 - CONTEXT matters!
- Read the axis labels!
 - **x-axis = independent variable**
 - **y-axis = dependent variable**



How to talk about graphs?

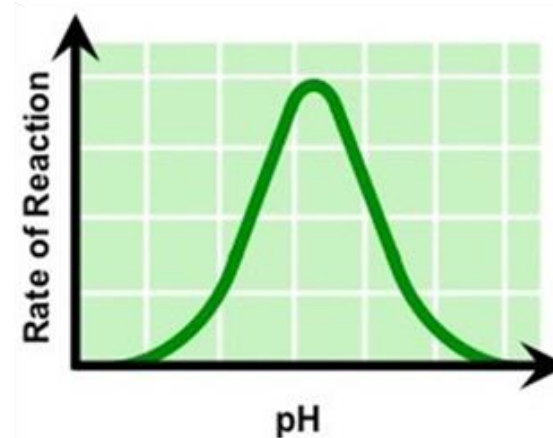
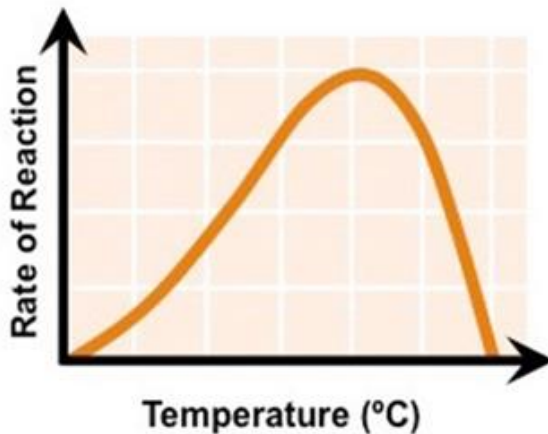
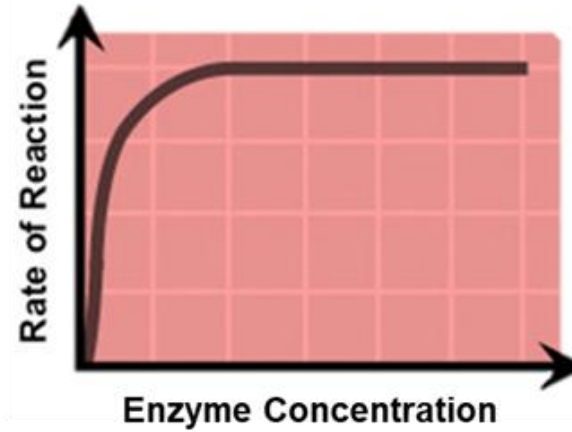
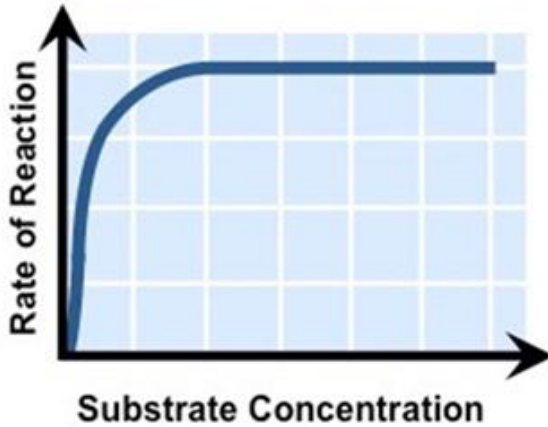
1. General trend

- First, describe how the independent variable and dependent variable changes **overall**
*E.g. when **x** increases, **y** increases then plateaus*
- If needed, **split the graph** into several parts to describe and/or explain separately

2. Comparative data quote

- Compare **2 points** to support your statement
- Provide the **x and y coordinates** with the correct **units**
- You can also quote the max and min values where appropriate
- You can include some manipulative figures
E.g. the number of cases reduced by half in 2010 compared to 2008

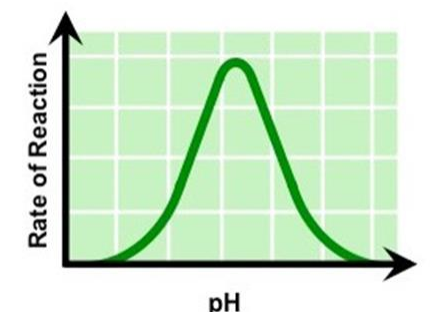
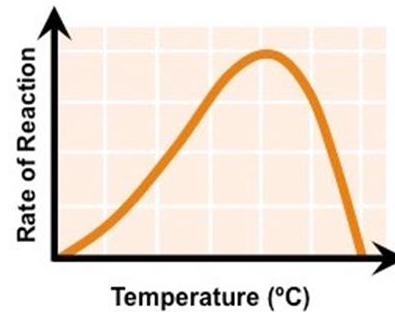
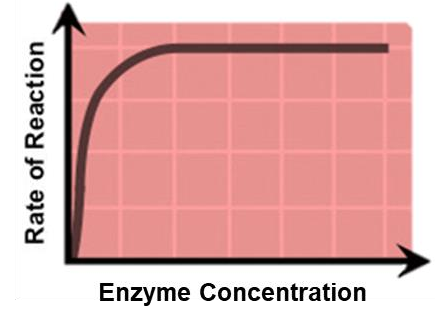
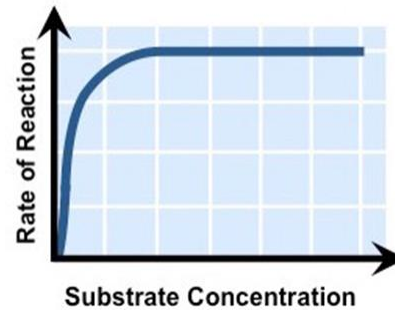
Factors Affecting Enzymatic Reaction



Rate of Reaction

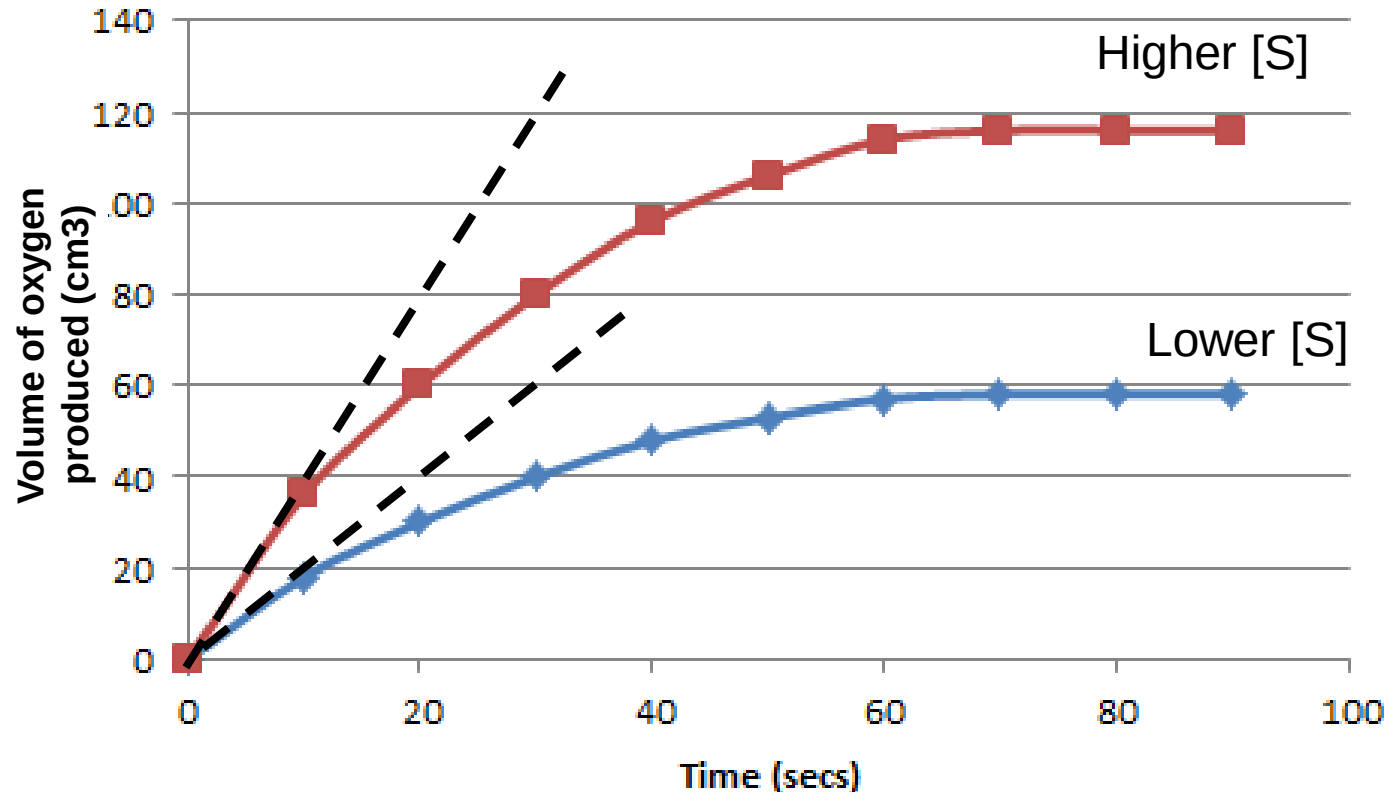
Rate of reaction be affected by:

1. Substrate concentration, $[S]$
2. Enzyme concentration, $[E]$
3. Temperature
4. pH
5. Inhibitors



1) Substrate Concentration

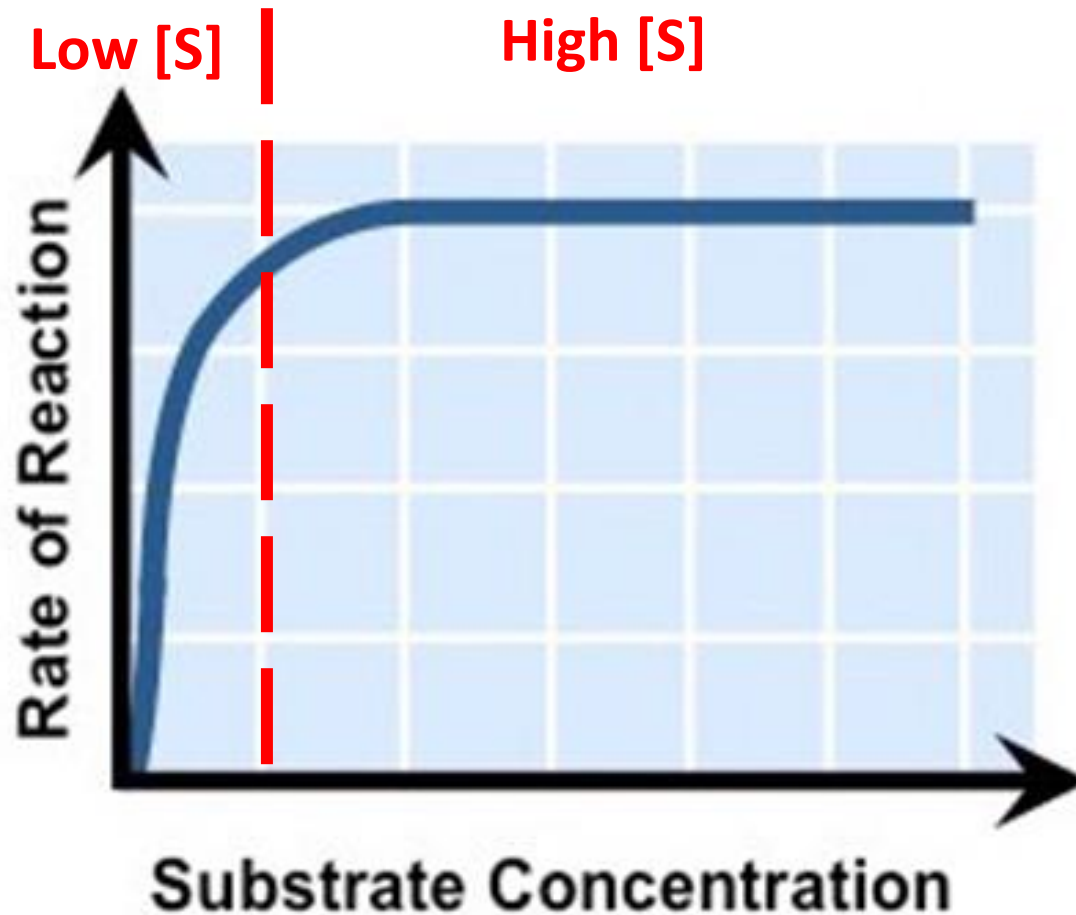
What would happen if we use a **higher [S]**?



How do we calculate the initial ROR?

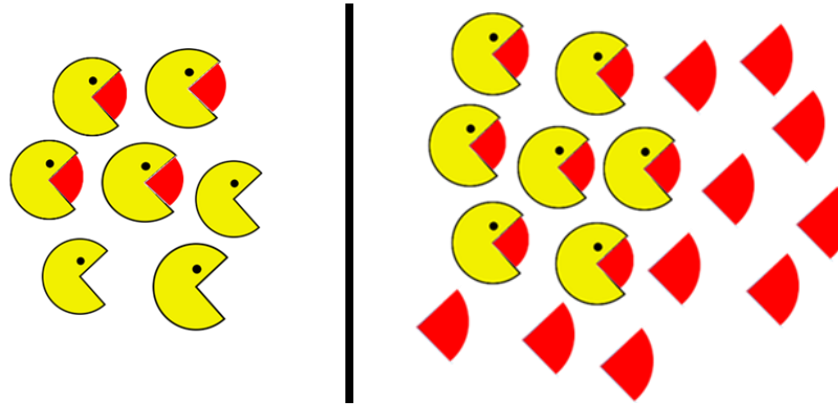
We will find that the reaction is faster / ROR is higher for the reaction with higher [S].

1) Substrate Concentration



[E] = constant

1) Substrate Concentration



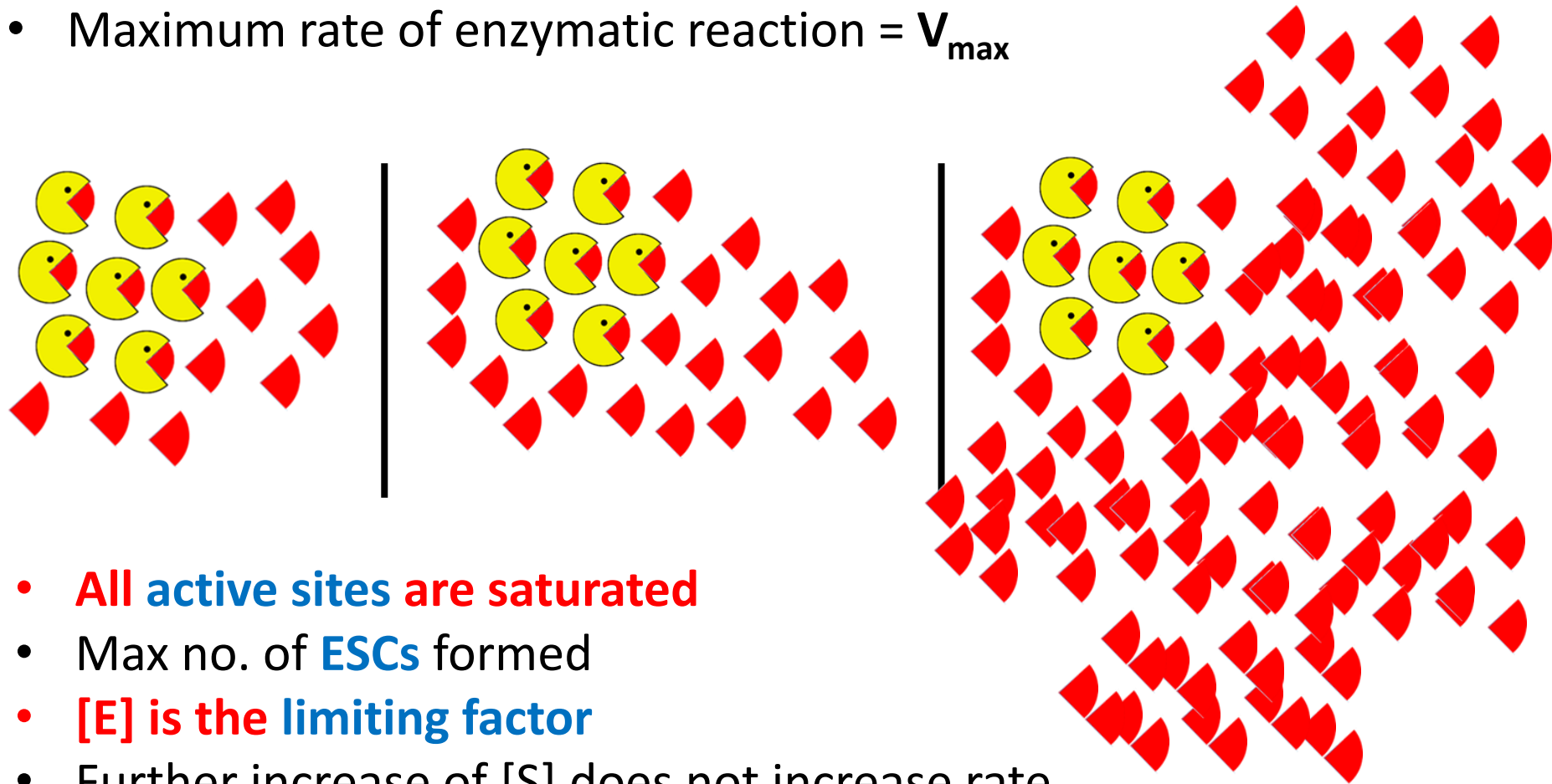
At low [S]

- Some **active sites** available for binding / not all occupied
- Few **collisions** between enzyme and substrate
- Less **S binds** with active site
- Few **ESCs** formed
- **[S] is limiting**
- **More active sites occupied as [S] increases**
- **Rate of reaction proportional to substrate concentration**

1) Substrate Concentration

At high [S]

- Rate increases to a plateau / $V_{\max}$ reached
- Maximum rate of enzymatic reaction = $V_{\max}$

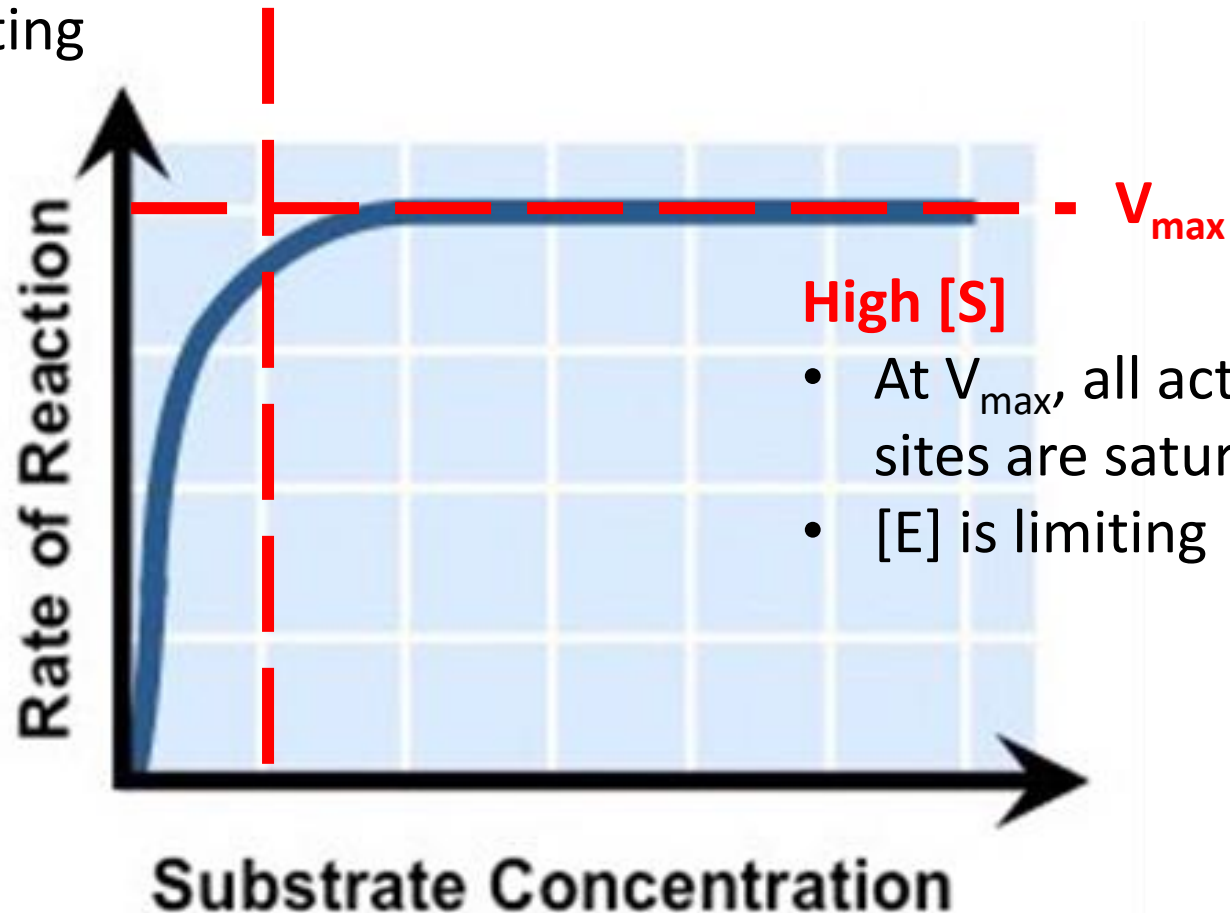


- All active sites are saturated
- Max no. of ESCs formed
- $[E]$ is the limiting factor
- Further increase of $[S]$ does not increase rate

1) Substrate Concentration

Low [S]

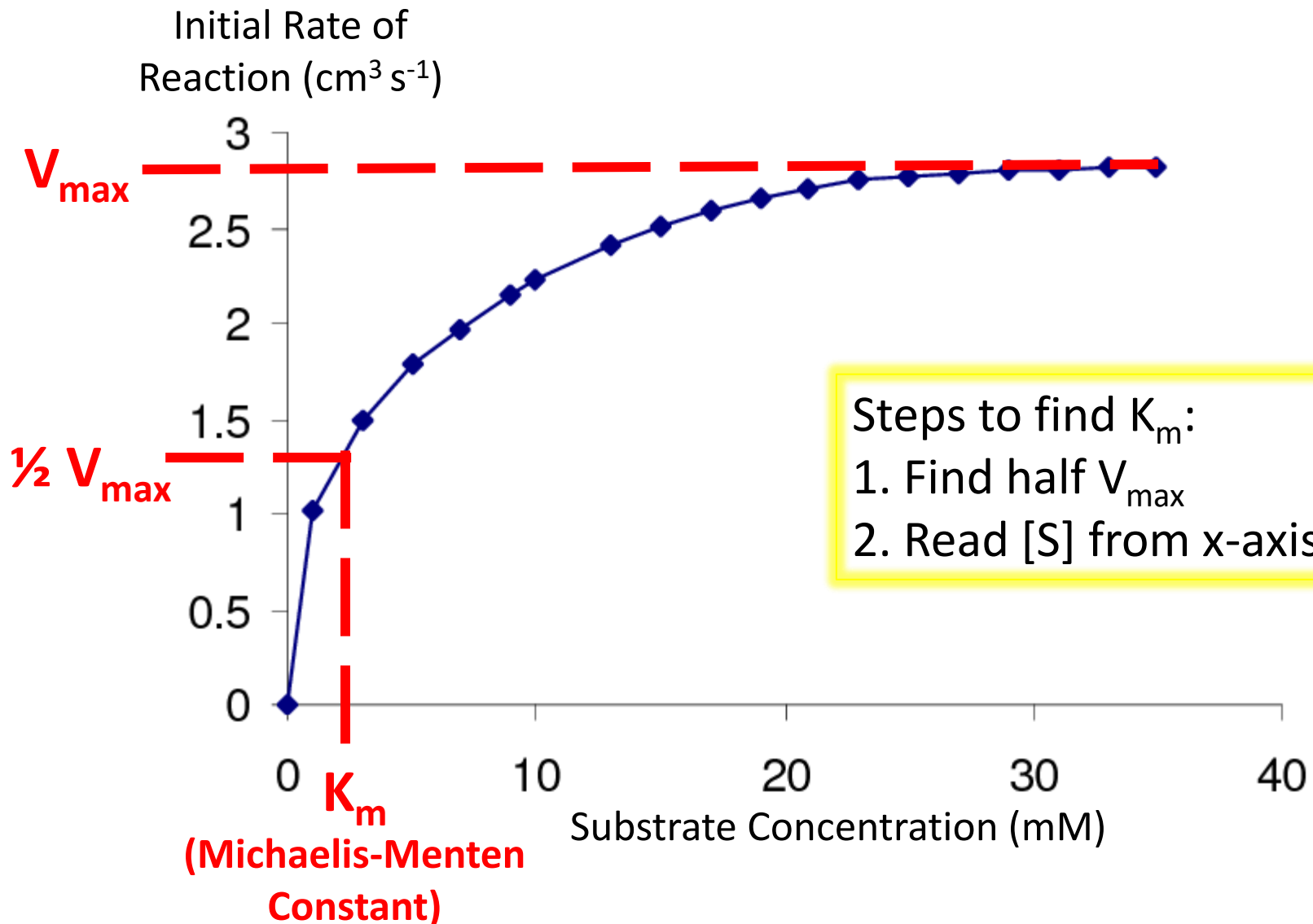
- Some active sites available
- [S] is limiting



High [S]

- At V_{max} , all active sites are saturated
- [E] is limiting

1) Substrate Concentration

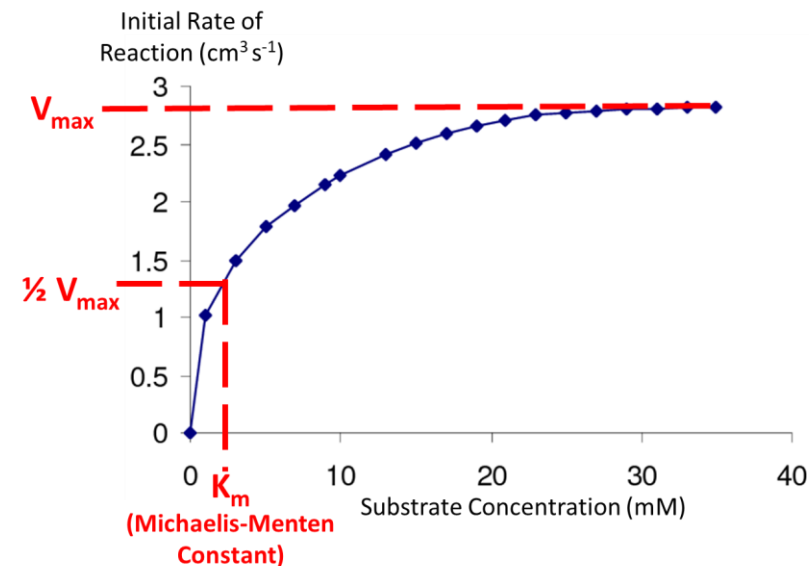


1) Substrate Concentration

Michaelis-Menten Constant (K_m)

- $K_m = [S]$ at $\frac{1}{2} V_{\max}$
- K_m = the $[S]$ at which the enzyme works at half its maximum rate
- At K_m , $\frac{1}{2}$ the active sites are occupied by S

- **K_m is a measure of enzyme affinity**
- **Affinity** = the degree of attraction between molecules



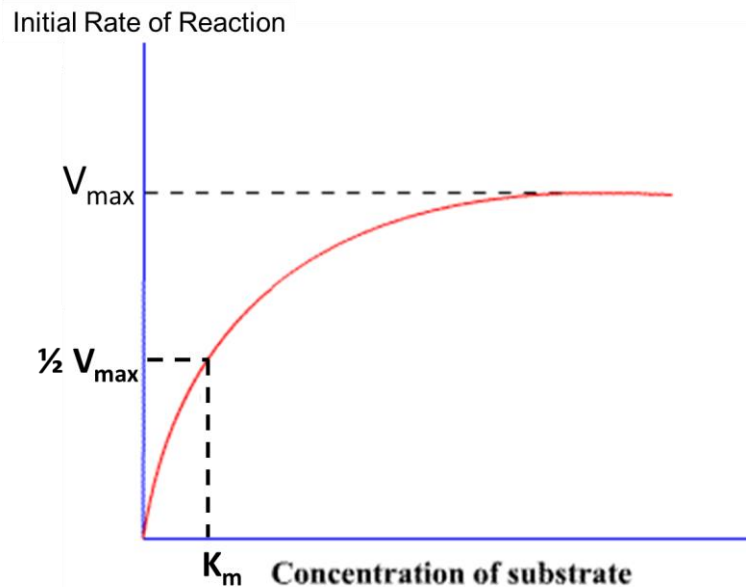
- **The higher the K_m , the lower the affinity**

1) Substrate Concentration

Michaelis-Menten Constant (K_m)

For an enzyme with high K_m and low affinity:

- **Higher $[S]$ is needed to reach $\frac{1}{2} V_{max}$**
- Enzyme needs a higher $[S]$ to reach V_{max}
- Enzyme forms fewer **ESC** in the same unit of time
- Enzyme **active site** is a less good fit for substrate



1) Substrate Concentration

Michaelis-Menten Constant (K_m)

TABLE 6-6 K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β -Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

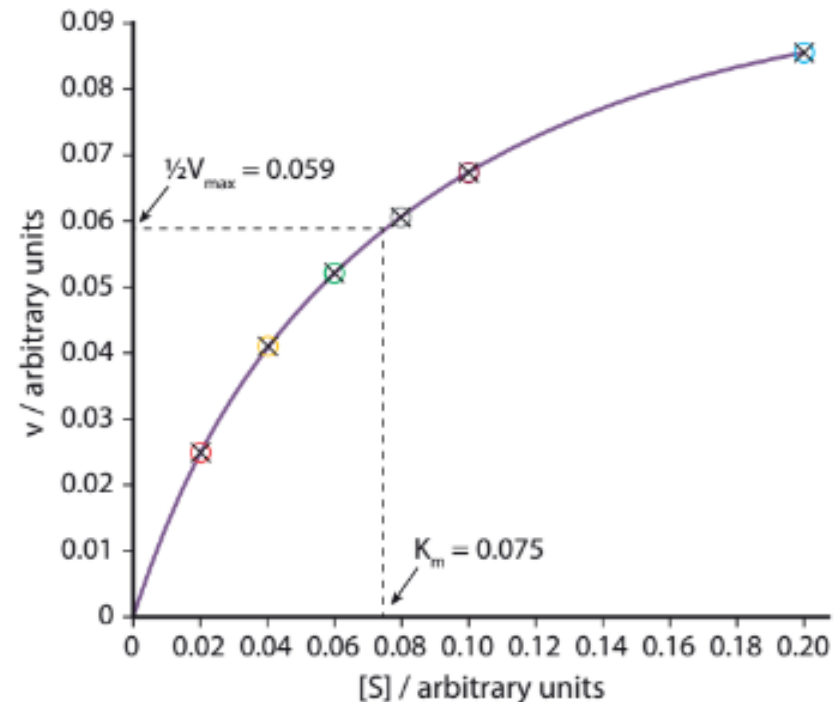
Table 6-6
Lehninger Principles of Biochemistry, Sixth Edition
© 2013 W. H. Freeman and Company

Question: Is brain hexokinase more likely to bind fructose or glucose?

1) Substrate Concentration

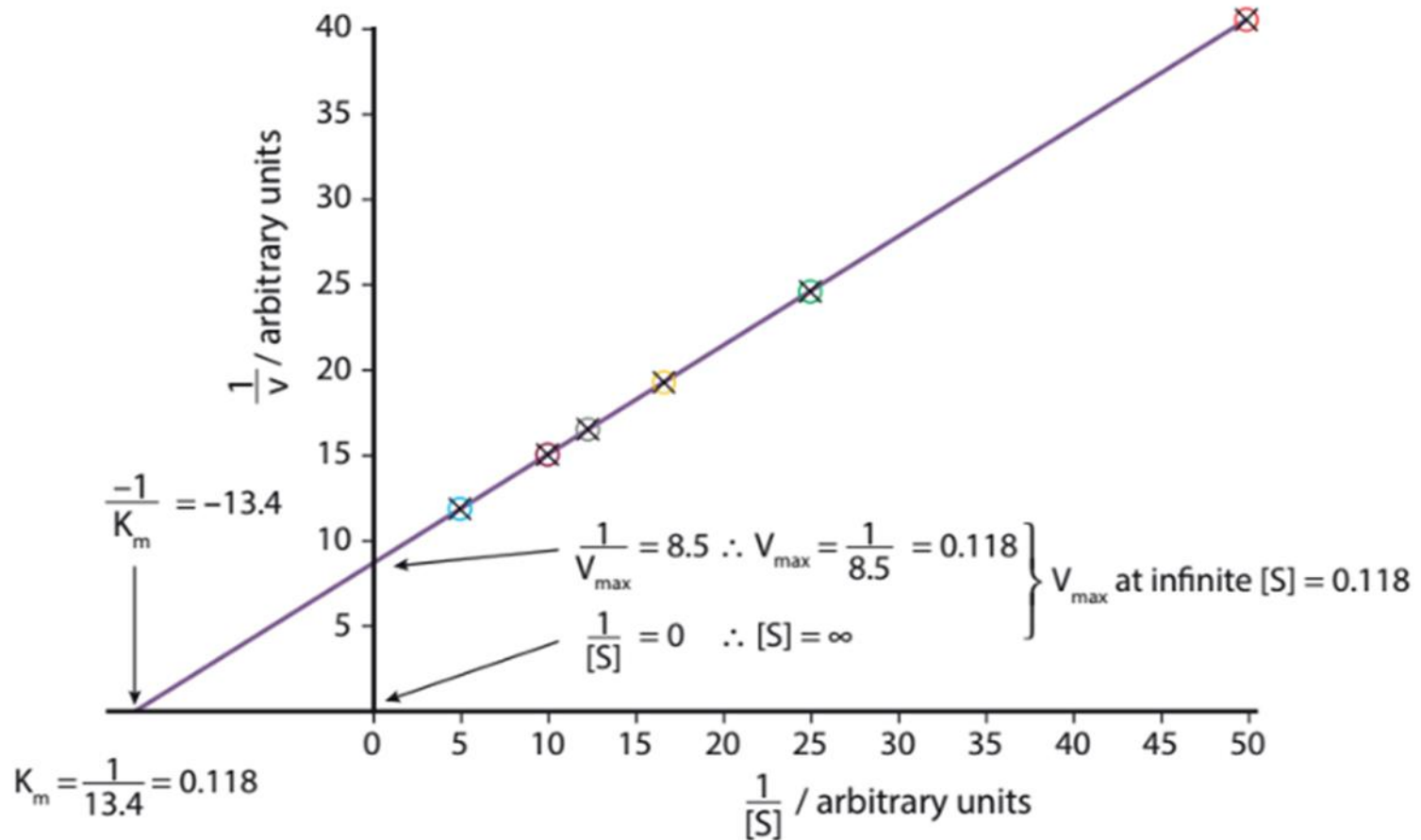
$$V_{\max} \text{ and } K_m$$

- Problem: too many experiments needed for the graph to plateau in order to find $V_{\max}$ and K_m
 - Solution: Plot a **double-reciprocal graph**!
- Plot **$1/v$ against $1/[S]$**
instead of v against $[S]$



1) Substrate Concentration

Double Reciprocal Plot

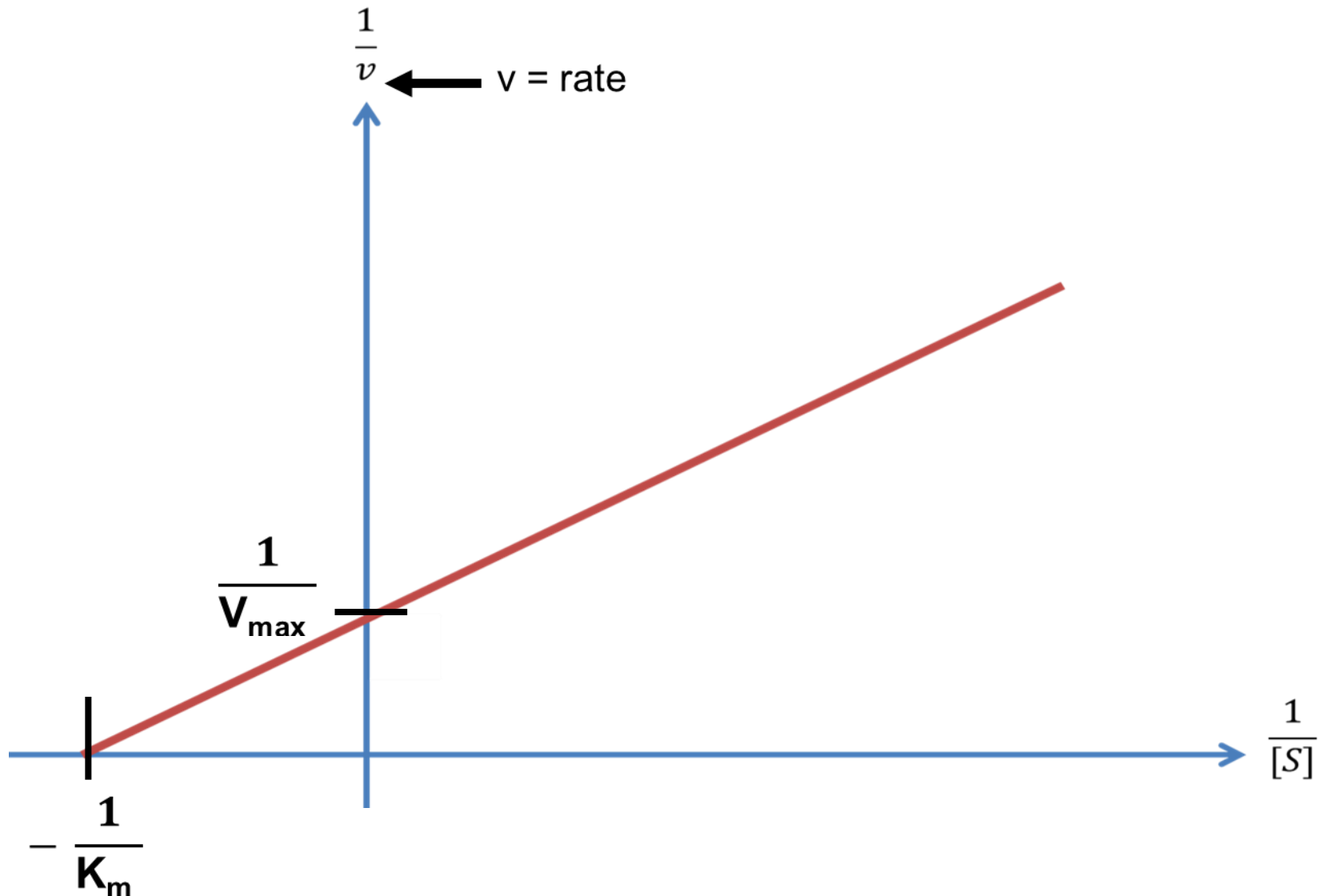


$$V_{\max} = 0.118 \text{ or } 0.1$$

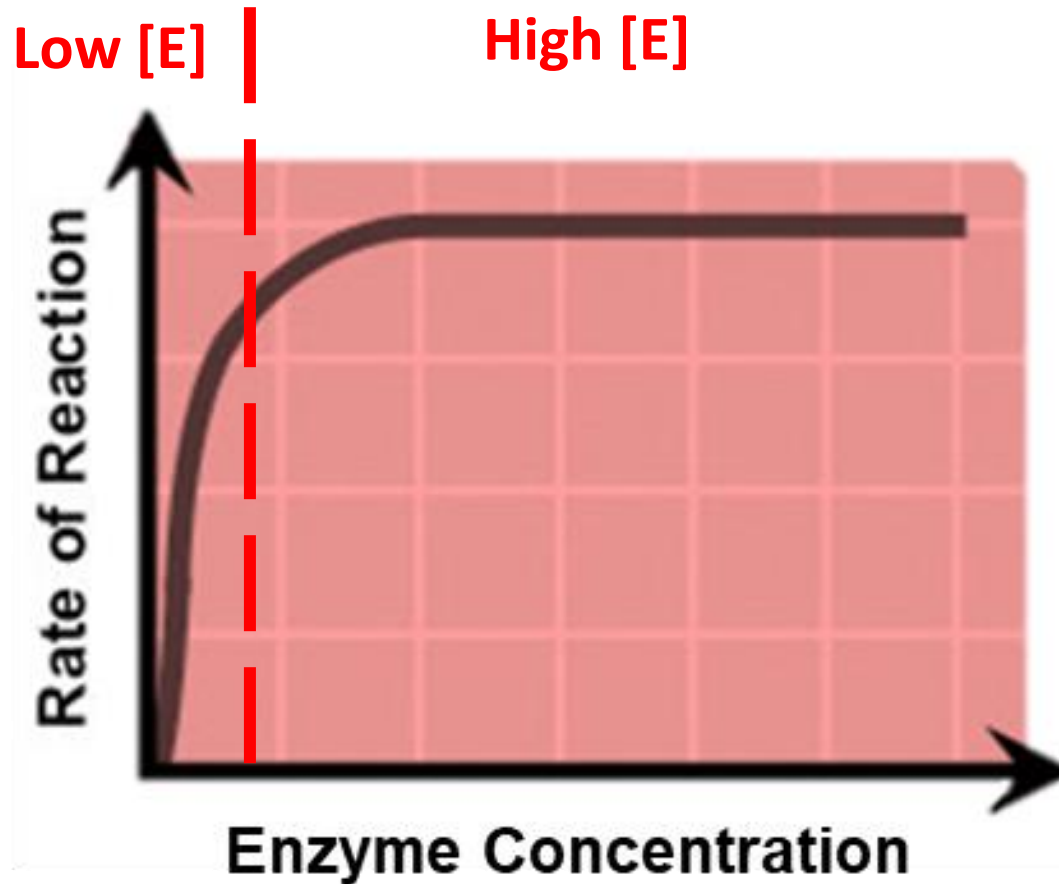
$$K_m = 0.07 \text{ or } 0.1$$

1) Substrate Concentration

Double-reciprocal plot



2) Enzyme Concentration



[S] = constant

2) Enzyme Concentration

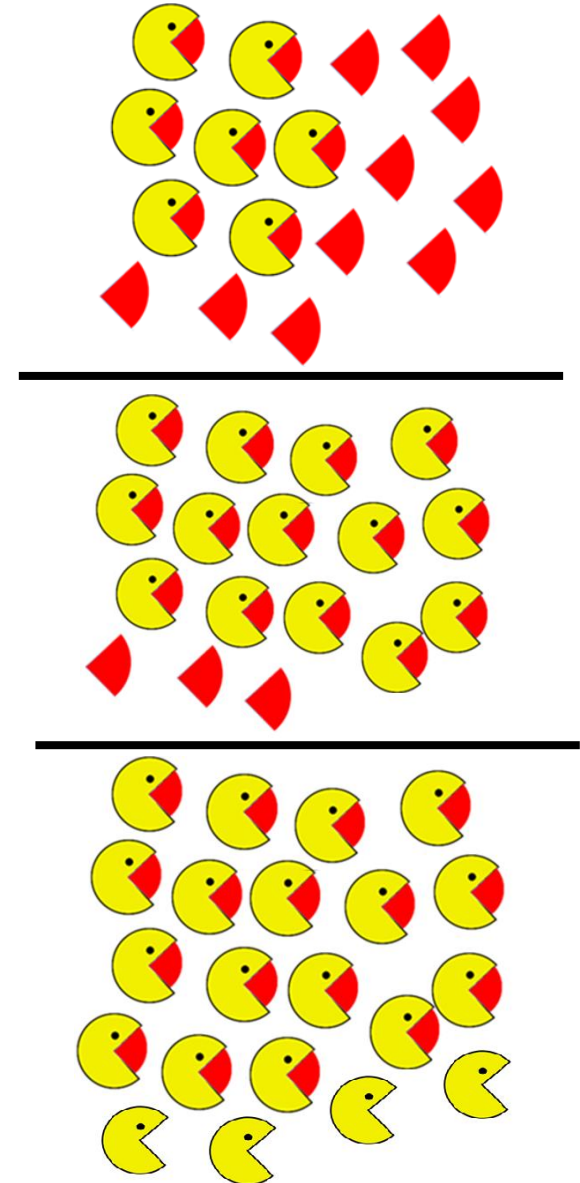
At low $[E]$, as $[E]$ increases:

- More enzymes present
- More **active sites** available for **S to bind**
- Increase in frequency of **collision**
- More **ESCs** form

→ **Rate increases as $[E]$ increases**

At high $[E]$:

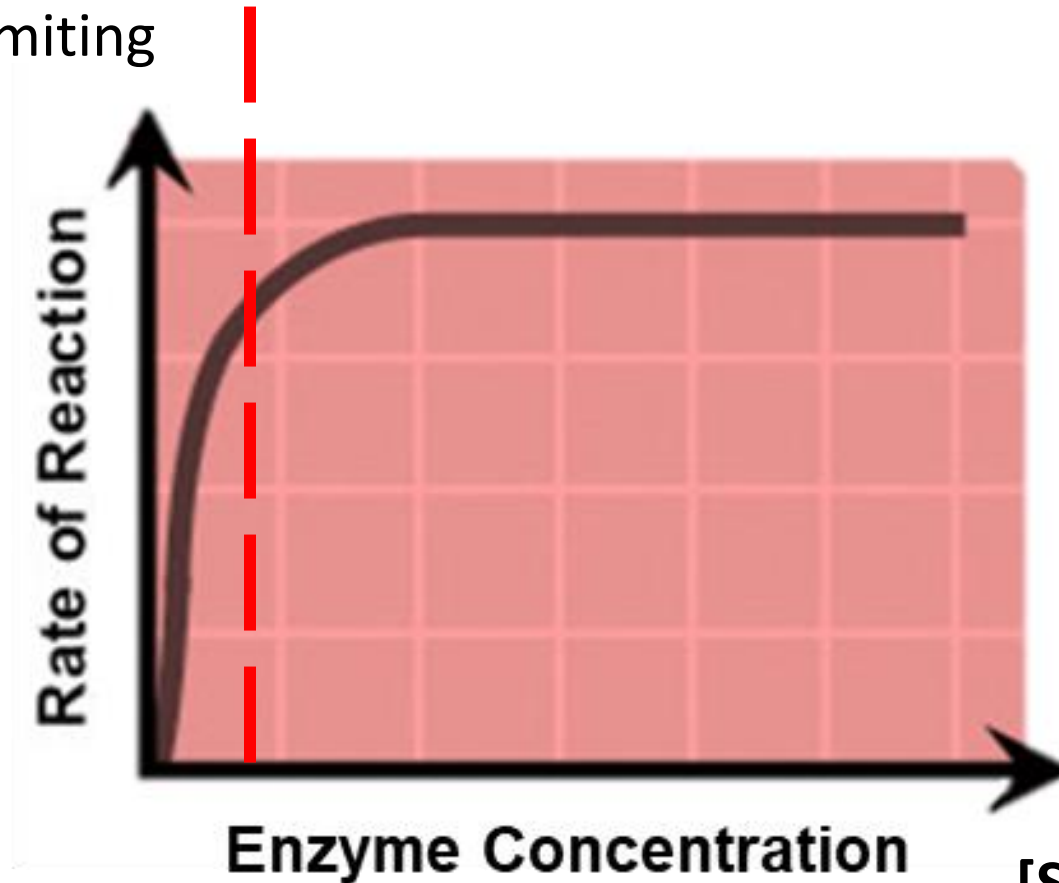
- Rate of reaction **levels off**
- **$[S]$ is the limiting factor**
- Max number of **ESCs** formed



2) Enzyme Concentration

Low [E]

- Less active sites available to bind to S
- [E] is limiting

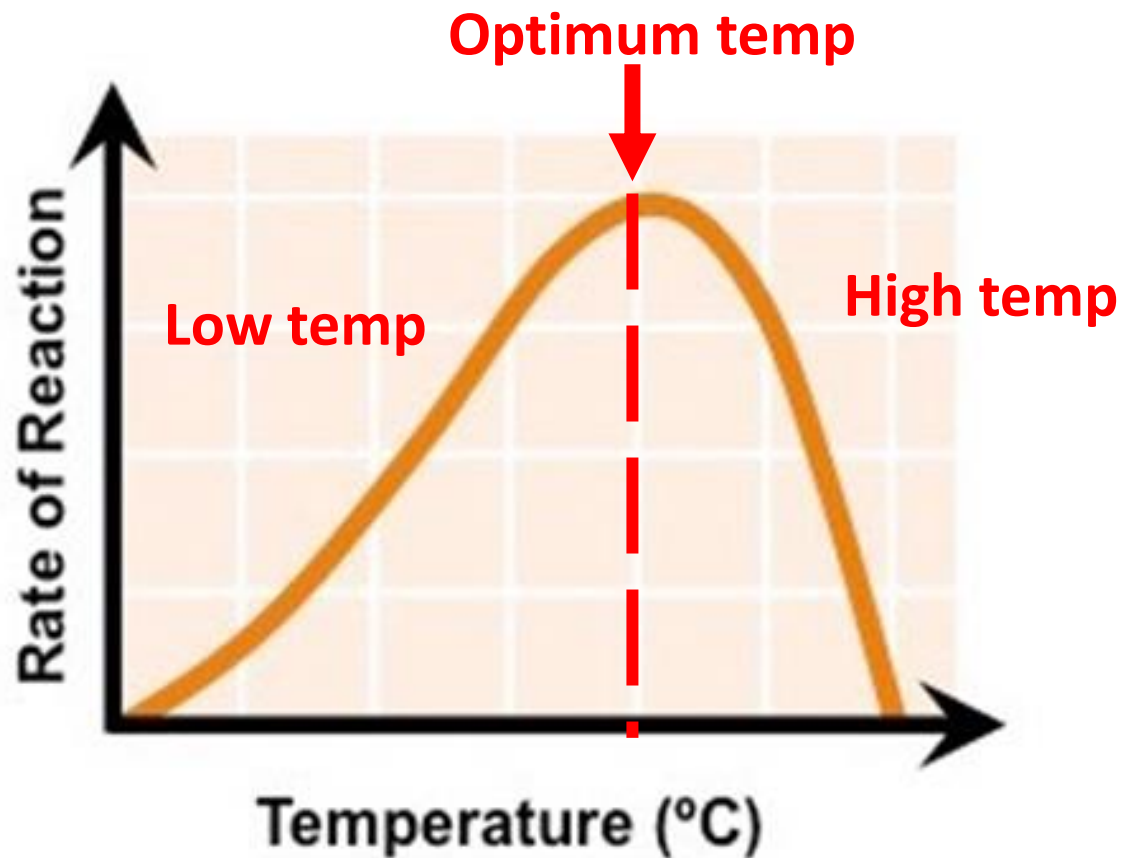


High [E]

- Max number of ESCs formed
- [S] is limiting

[S] = constant

3) Temperature

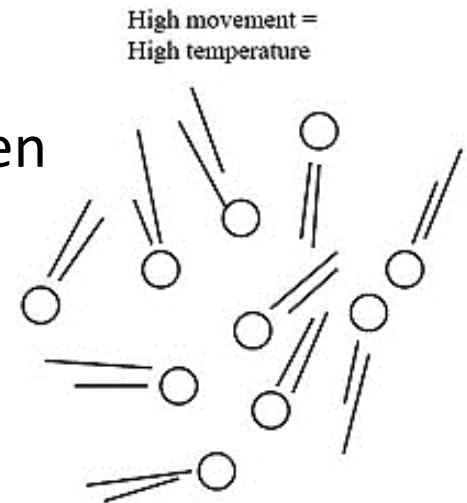
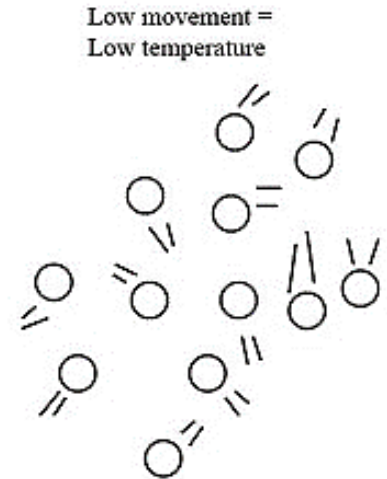


[S] = constant
[E] = constant

3) Temperature

At low temp, as temp increases from low to optimum:

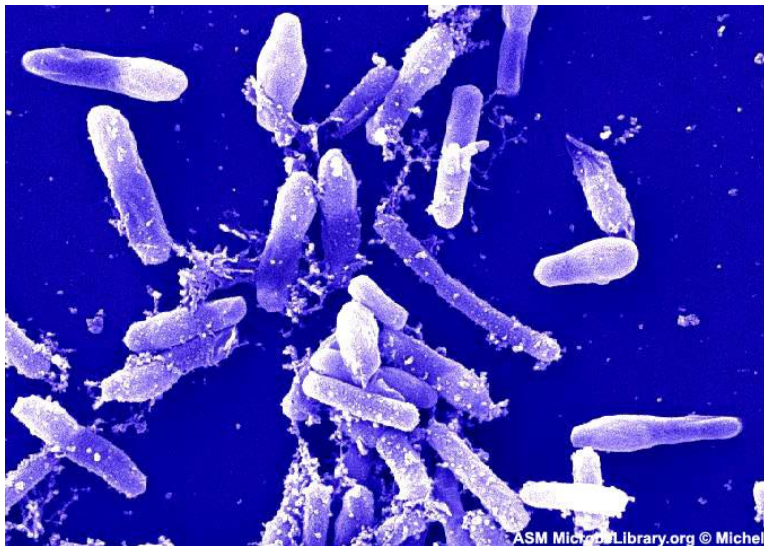
- Increase in **kinetic energy**
 - Enzyme and substrate molecules move **faster**
 - Increase in **collision** rate between substrate molecules and enzyme's active site
 - **Substrates bind** to enzyme's **active site** more often
 - More **ESCs** form
- **Rate increases as temp increases**



3) Temperature

At optimum temperature:

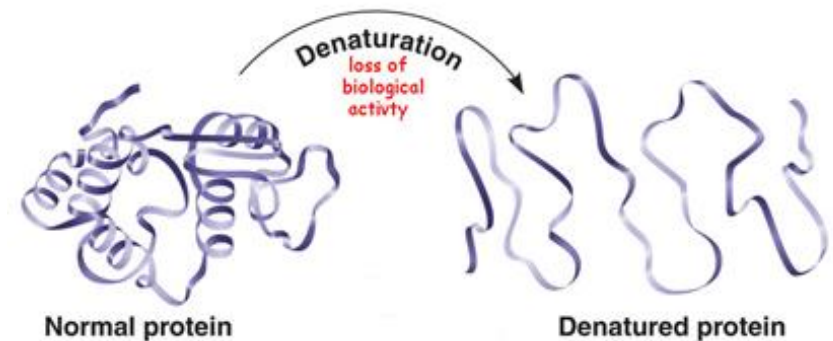
- **Maximum rate of enzyme reaction**
- Most human enzymes $\sim 40^{\circ}\text{C}$
- Different enzymes, different optimum temperature
E.g. enzymes in Thermophilic archaea have very high optimum temp



3) Temperature

At very high temp:

- **Reaction slows down and then stops**
- **H bonds and ionic bonds** holding enzyme start to break
- **Active site changes shape**
- Enzyme is **denatured**
- **Substrate cannot bind** to the **active site**
- Fewer **ESCs** form
- Irreversible

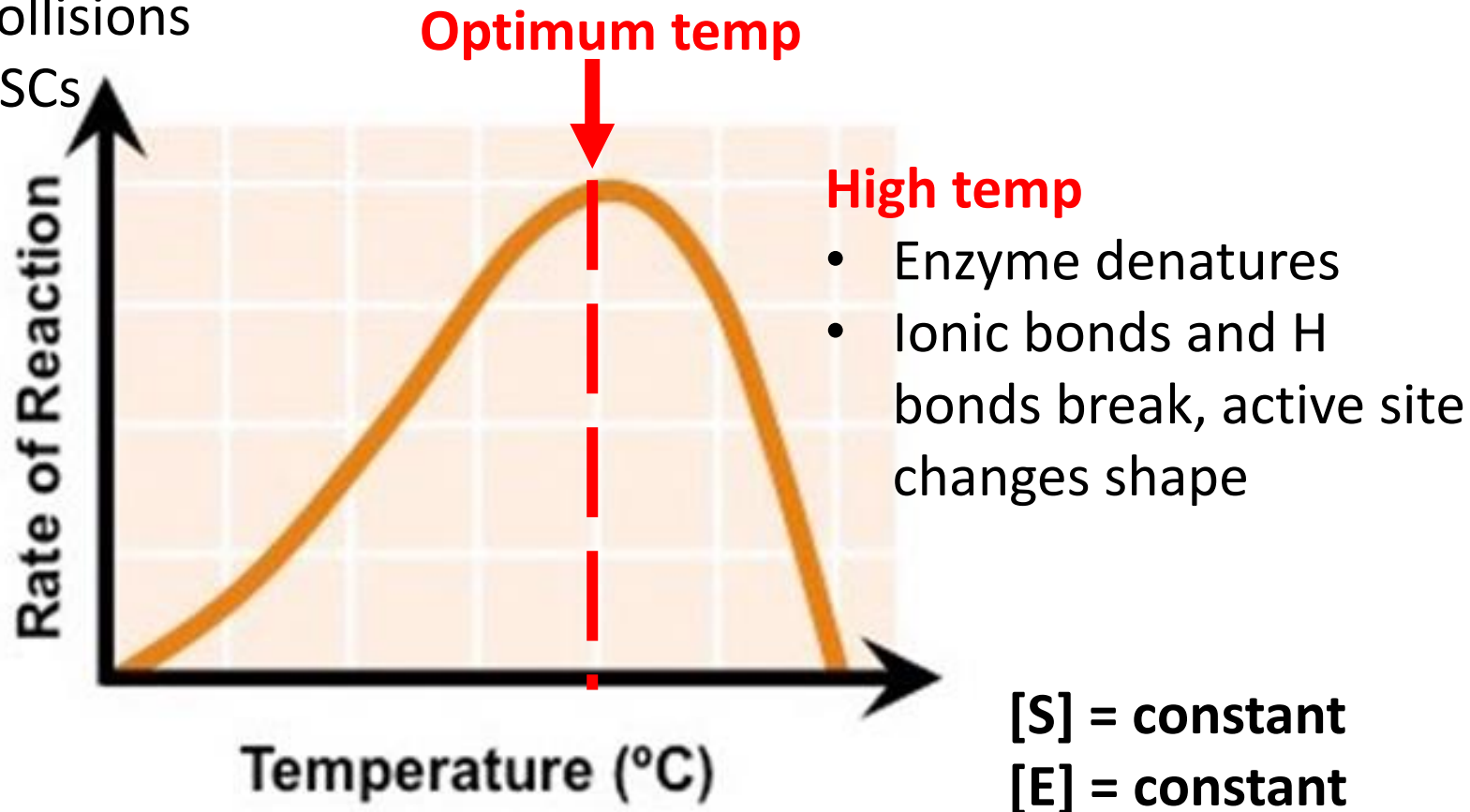


You're so **hot**
that you
denature my
enzymes.

3) Temperature

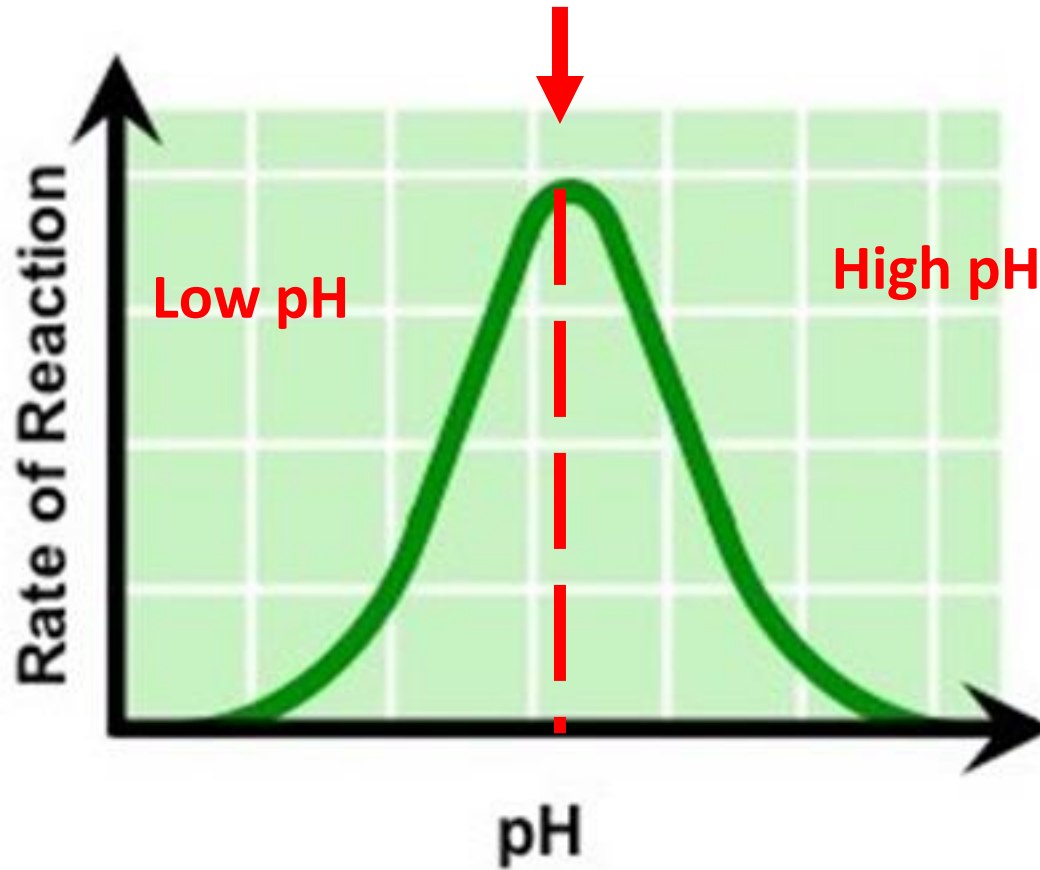
Low temp

- As temp increases, kinetic energy increases
- More collisions
- More ESCs



4) pH

Optimum pH



[S] = constant

[E] = constant

4) pH

- pH = measure of the concentration of **hydrogen ions** in a solution
- Low pH – acidic (more $[H^+]$, less $[OH^-]$)
- High pH – alkaline (less $[H^+]$, more $[OH^-]$)

At low/high pH:

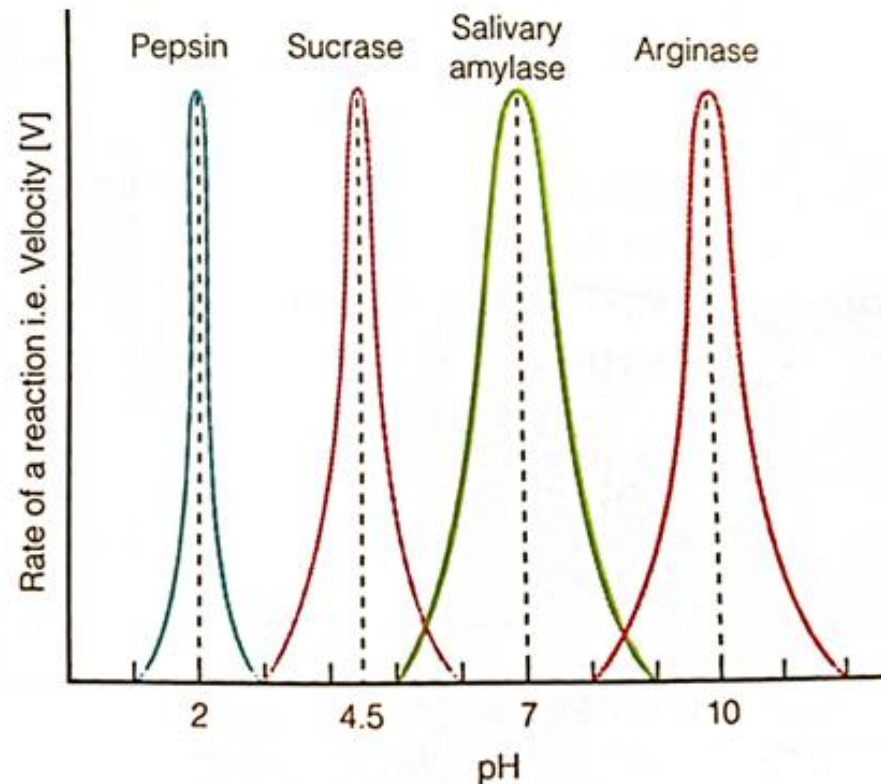
- H^+ or OH^- can interact with the R groups of amino acids in enzymes
- Disrupt **ionic bonds and H bonds**
- Changes the **active site** shape
- **S cannot bind** to active site
- Less **ESCs** form



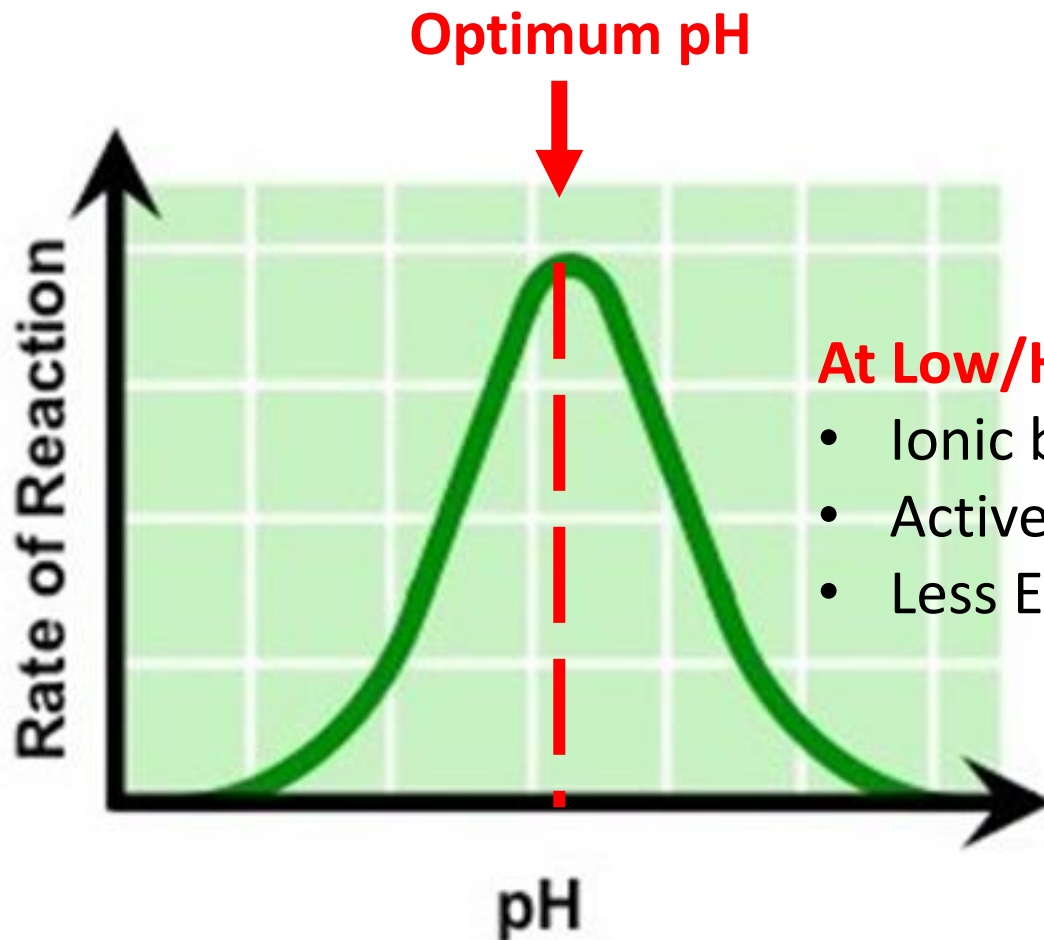
4) pH

At optimum pH:

- **Max rate of enzyme reaction**
- Beyond optimum pH - Enzyme denatures
- Different enzymes, different optimal pH



4) pH



At Low/High pH:

- Ionic bonds and H bonds break
- Active site change shape
- Less ESCs form

[S] = constant

[E] = constant

5) Inhibitors

- Molecules which can reduce rate of an enzyme catalysed reaction

Places where inhibitors can bind:-

1) Bind to enzyme's **active site**

- Similar to substrate's shape
- **Competitive inhibitor**

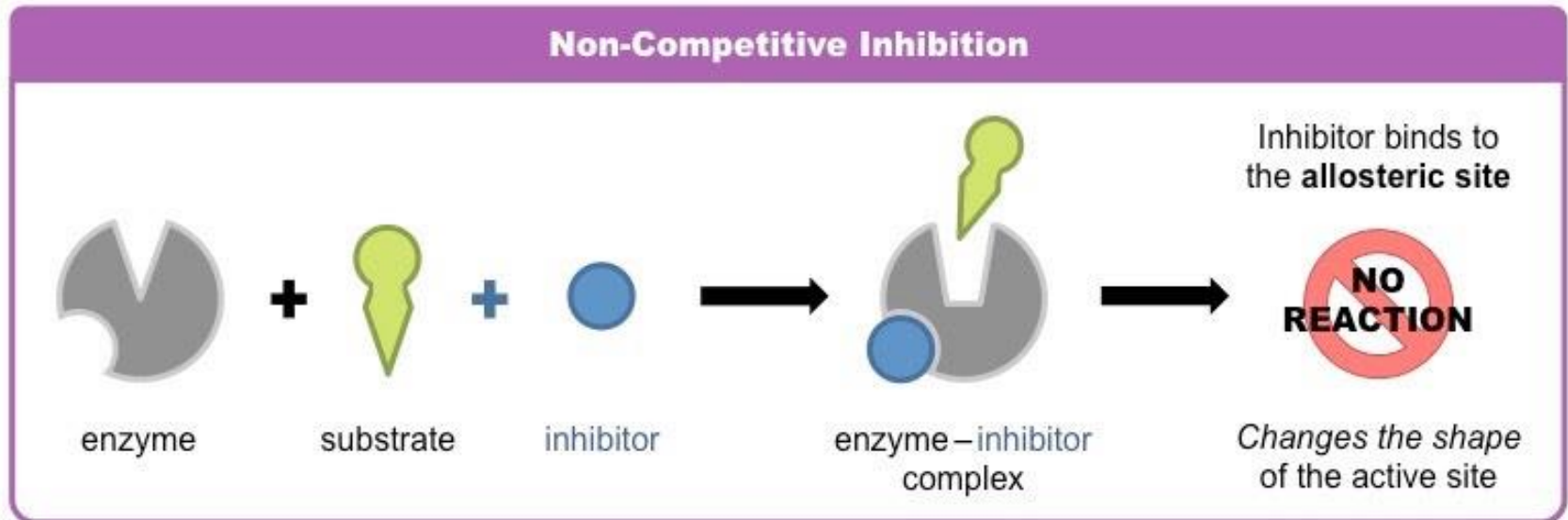
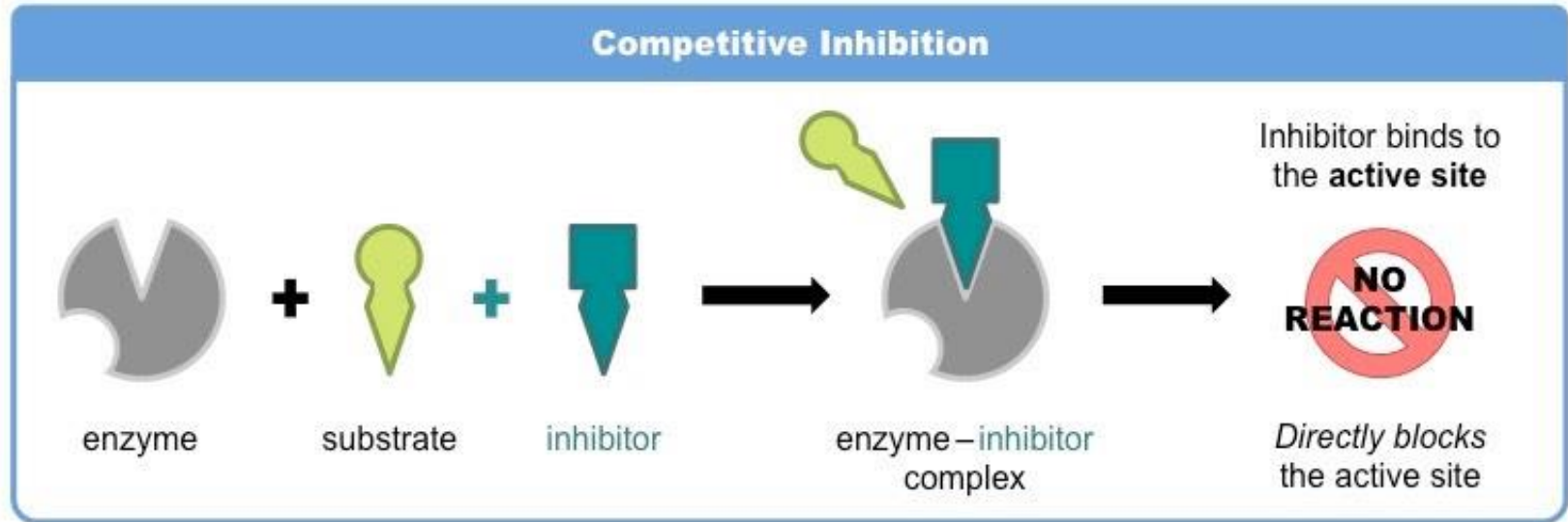
2) Bind to **allosteric site** of enzyme

- Another site on enzyme other than the active site
- **Non-competitive inhibitor**



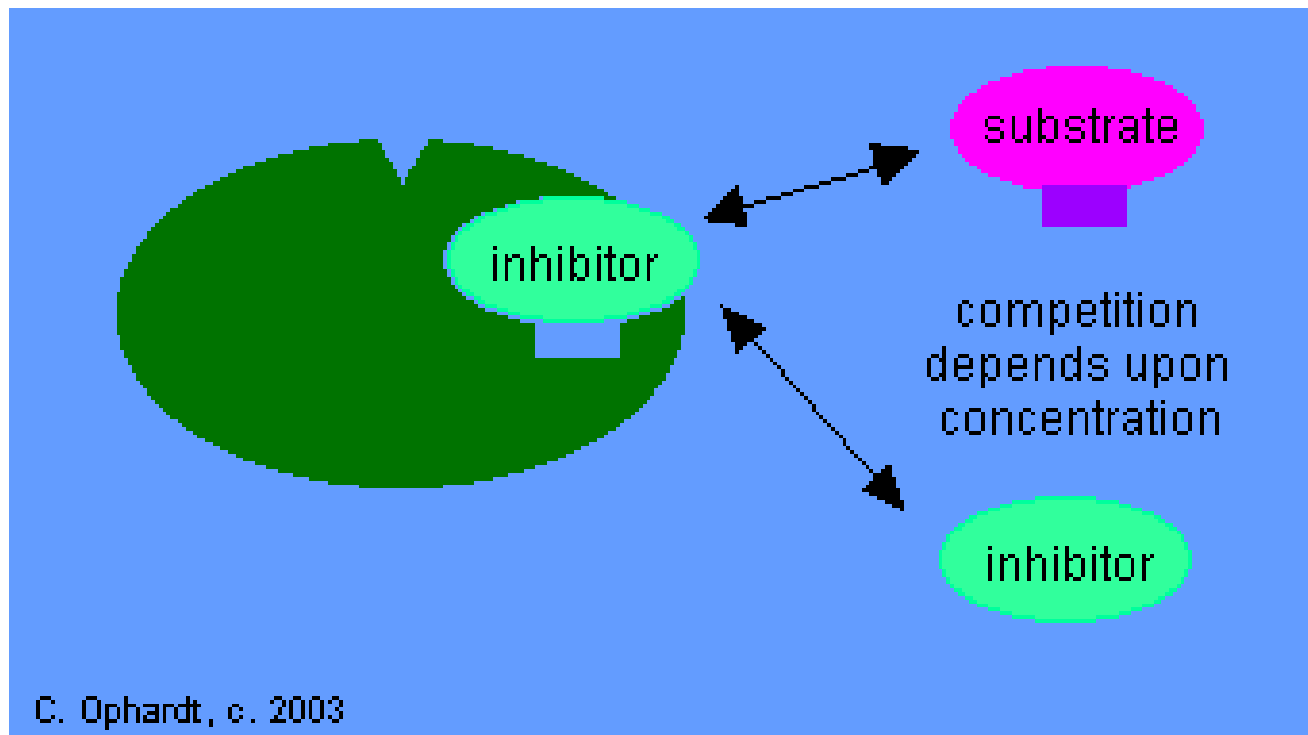
5) Inhibitors

- Two types:



Competitive Inhibitors

- Fit into enzyme's active site
- **Similar shape** to real substrate
- Competitive inhibitor **competes with substrate for active site** of enzyme
- **Reversible**

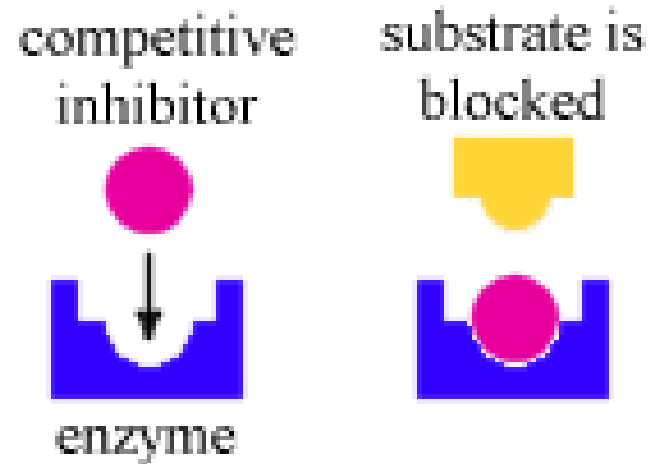


Competitive Inhibitors

At low [S]:

- $[\text{inhibitor}] > [\text{substrate}]$
- Less frequency of **collisions** with S
- Less **ESCs** forms
- Reduces rate of reaction

→ Enzyme's function **inhibited**



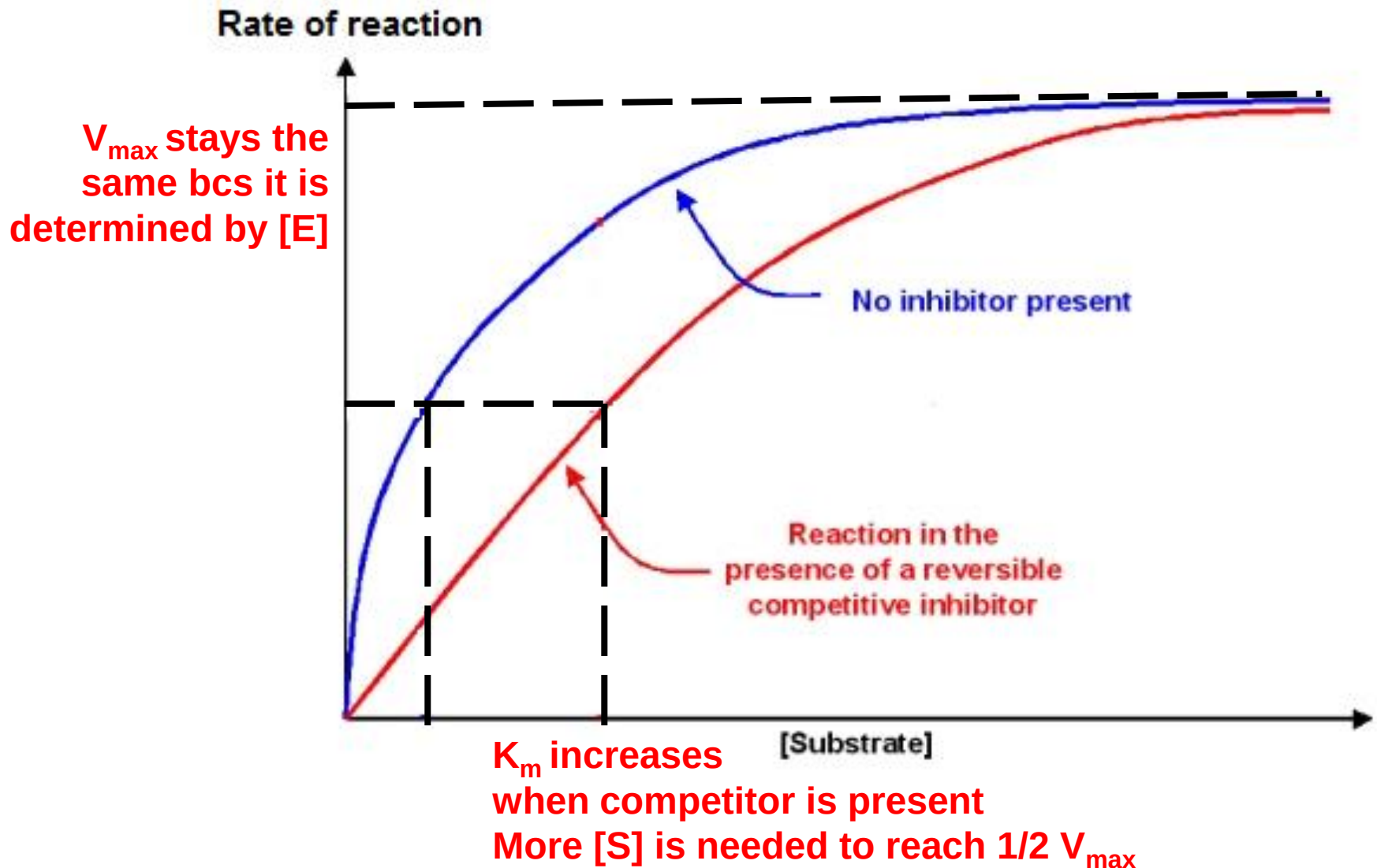
Competitive Inhibition

- Can be reversed/overcome by.... having more substrates

At high [S]

- $[S] > [\text{inhibitors}]$
- Inhibitor has less effect
- **Substrate outcompetes inhibitor** at high [S]
- More chances of substrate molecule **colliding** and binding to **active site**
- More **S binds** to active site
- More **ESCs** form
- Enzyme's function **unaffected**
- **Competitive, reversible inhibition**

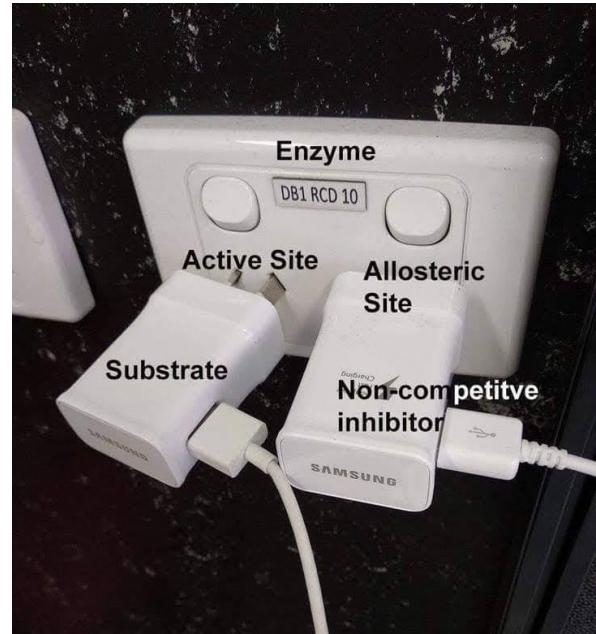
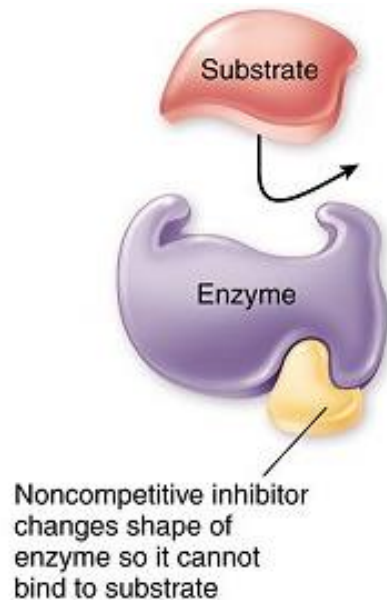
Competitive Inhibition



Non-competitive inhibition

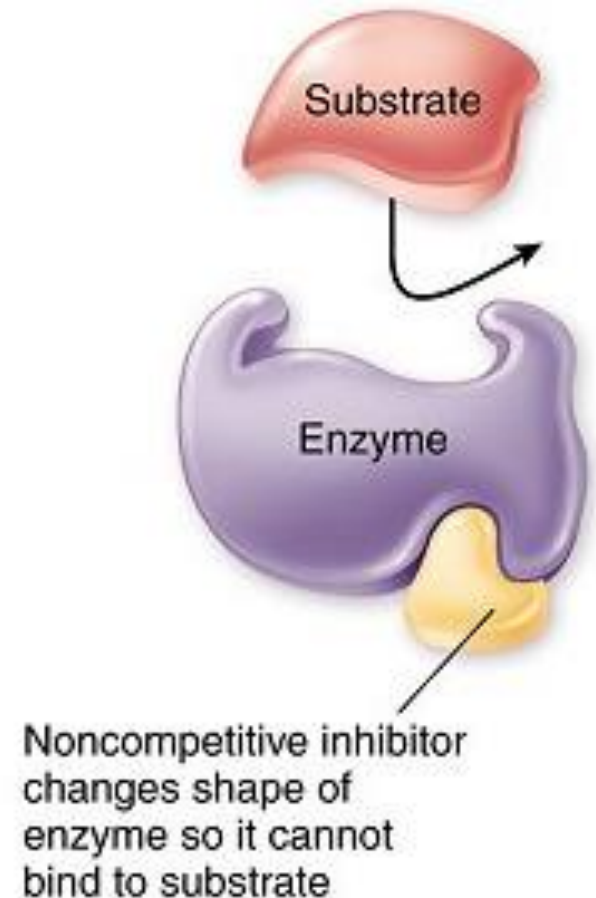
Two types of non-competitive inhibition:

- Irreversible – e.g. in poisons (cyanide binds to enzyme in mitochondria and inhibits ATP synthesis)
- Reversible – end product inhibition

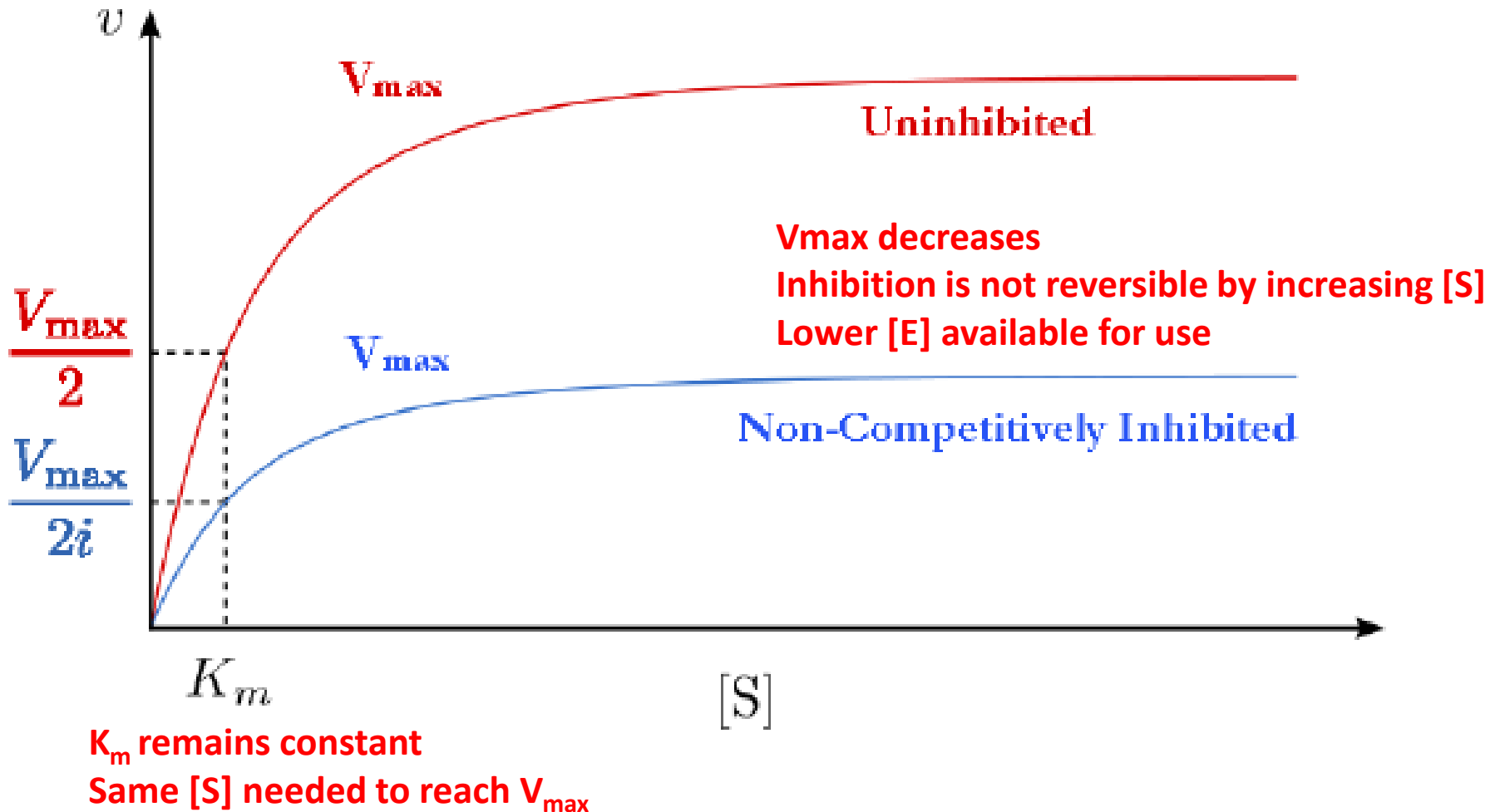


Irreversible Non-Competitive Inhibition

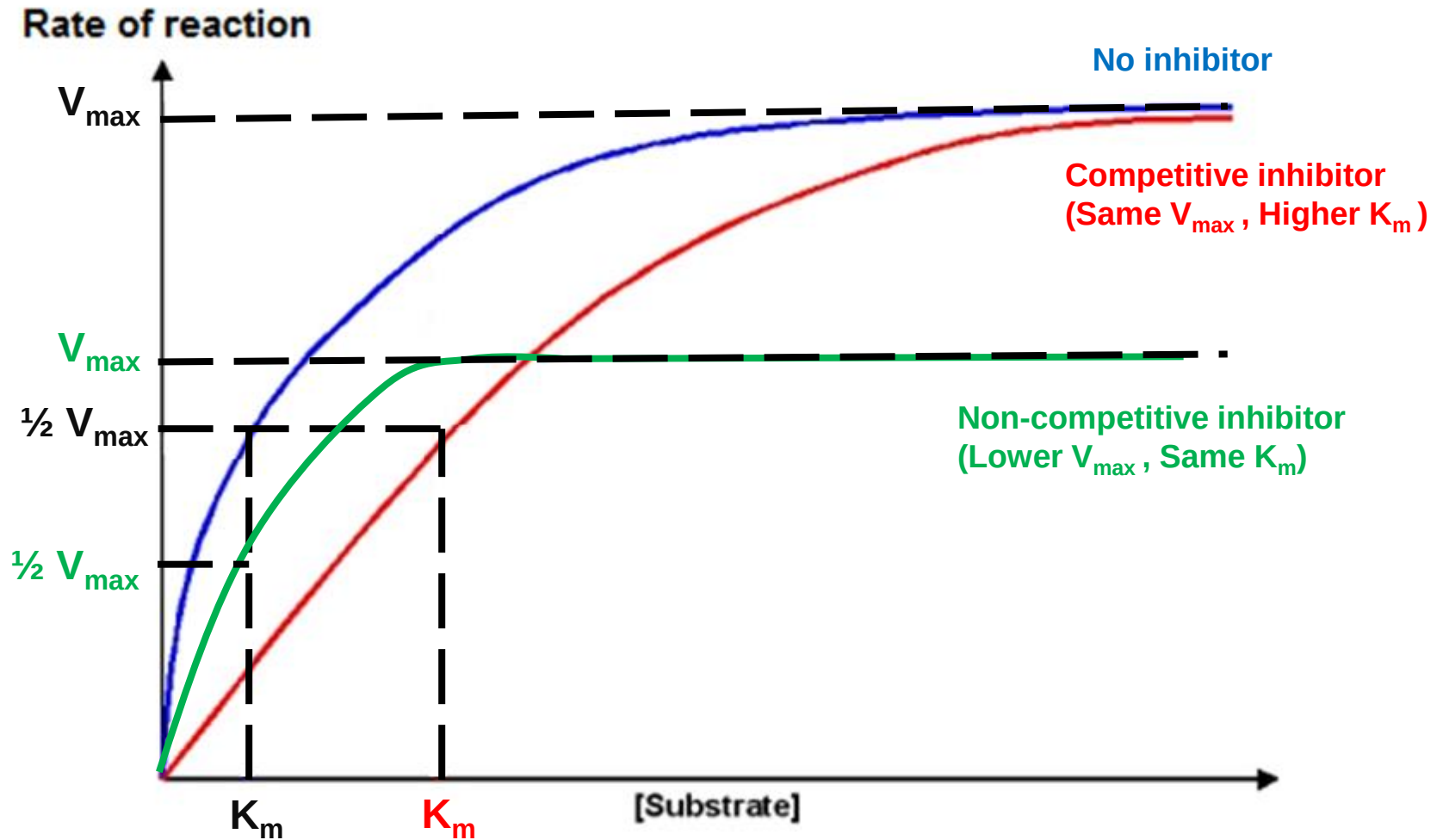
- Inhibitor binds to **allosteric site** on enzyme
- **Allosteric site** = another site on enzyme other than the active site
- **Changes active site's shape**
- Disrupts H bonds and hydrophobic interactions
 - 3D shape of enzyme affected
 - Distortion ripples
- **Substrate unable to bind to active site**
 - Enzyme's function - inhibited
- Increasing substrate concentration has no effects on enzyme activity



Irreversible Non-Competitive Inhibition



Graphs

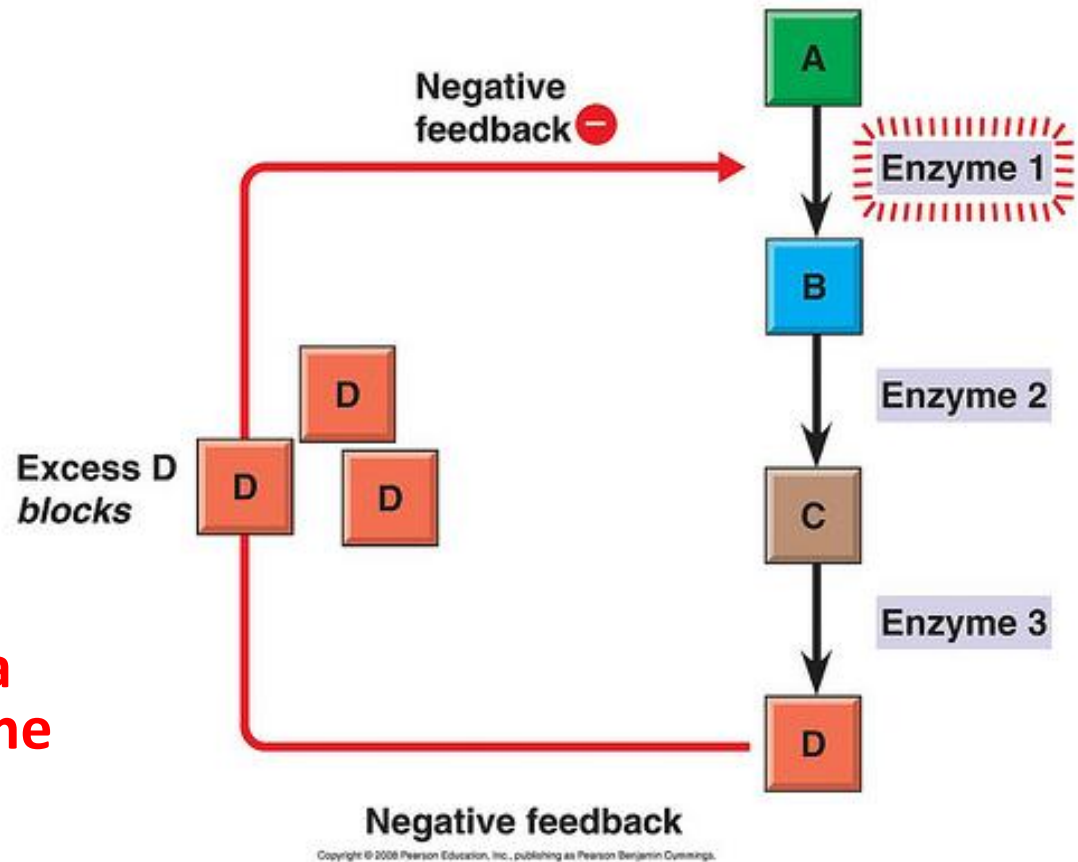


End Product Inhibition

- A form of control for metabolic reactions
- Maintain homeostasis

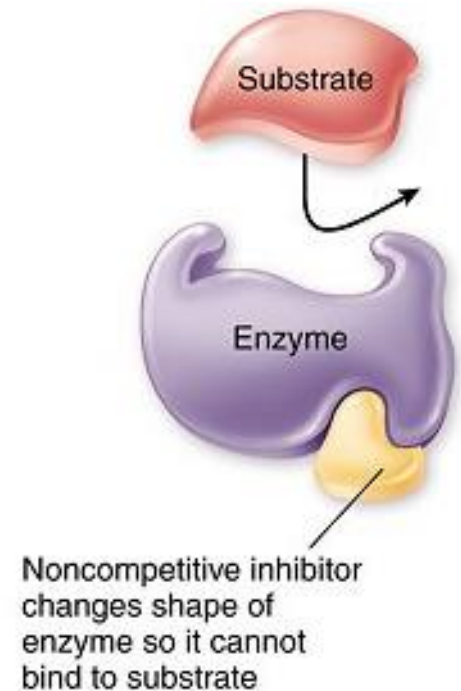
In a chain of reactions

- Catalyzed by various enzymes
- **End product becomes a non-competitive enzyme inhibitor for upstream reactions**

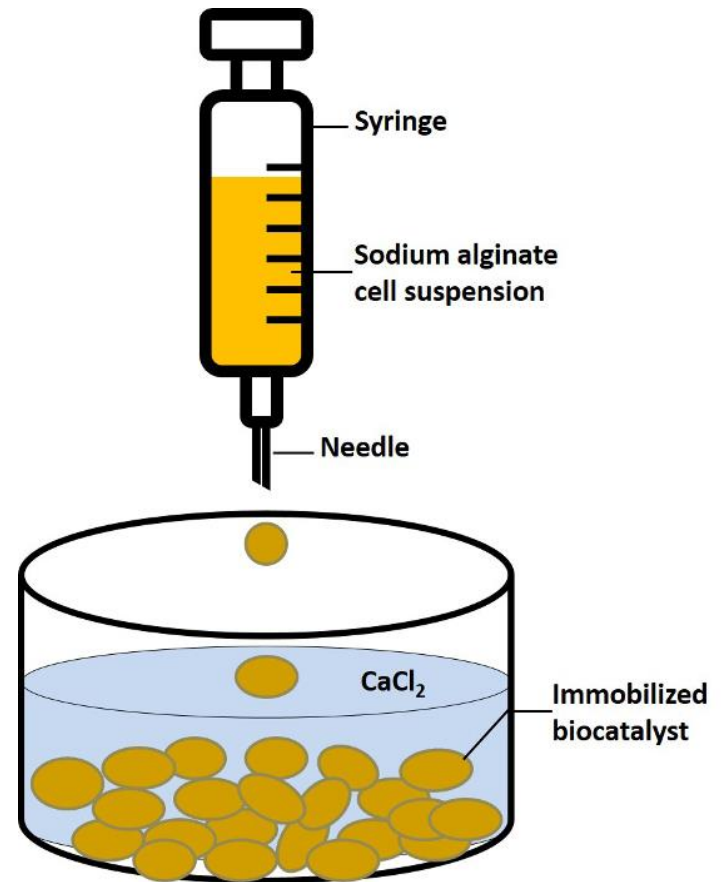


End Product Inhibition

- High amount of end product
 - binds to **allosteric site** on another enzyme catalyzing a reaction in the same metabolic chain
 - Inhibits reaction
- **When amount of end product (inhibitor) decreases, rate of reaction increases again**
- Inhibitors can lose its attachment on enzyme's allosteric site and are used elsewhere
 - Temporary/brief
 - Non-competitive, **reversible** inhibition

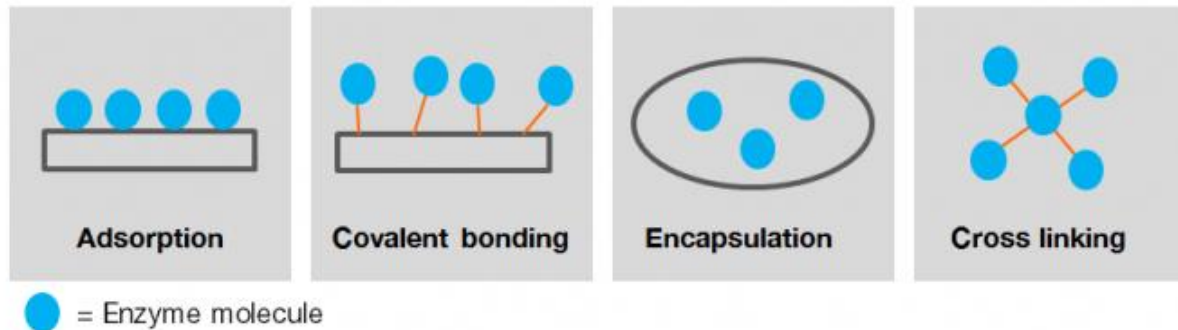


Immobilised Enzymes



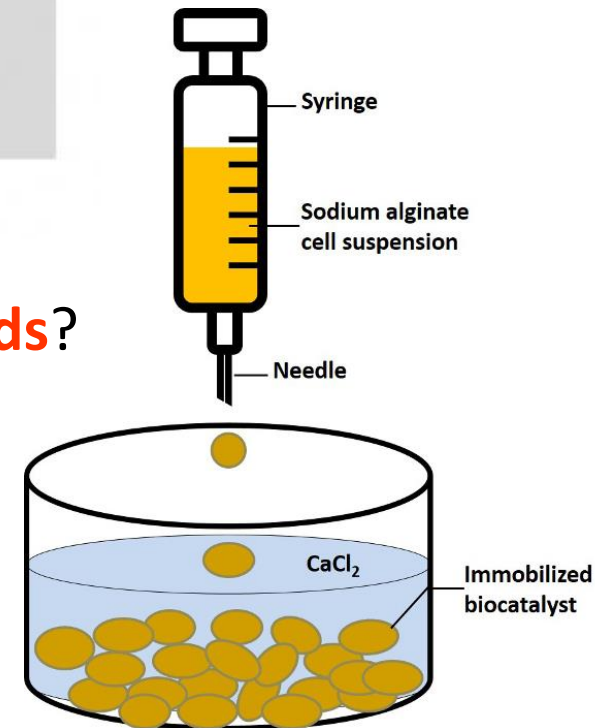
Immobilised Enzymes

- Enzymes attached to an inert, insoluble material



How to **encapsulate enzymes** in **alginate beads**?

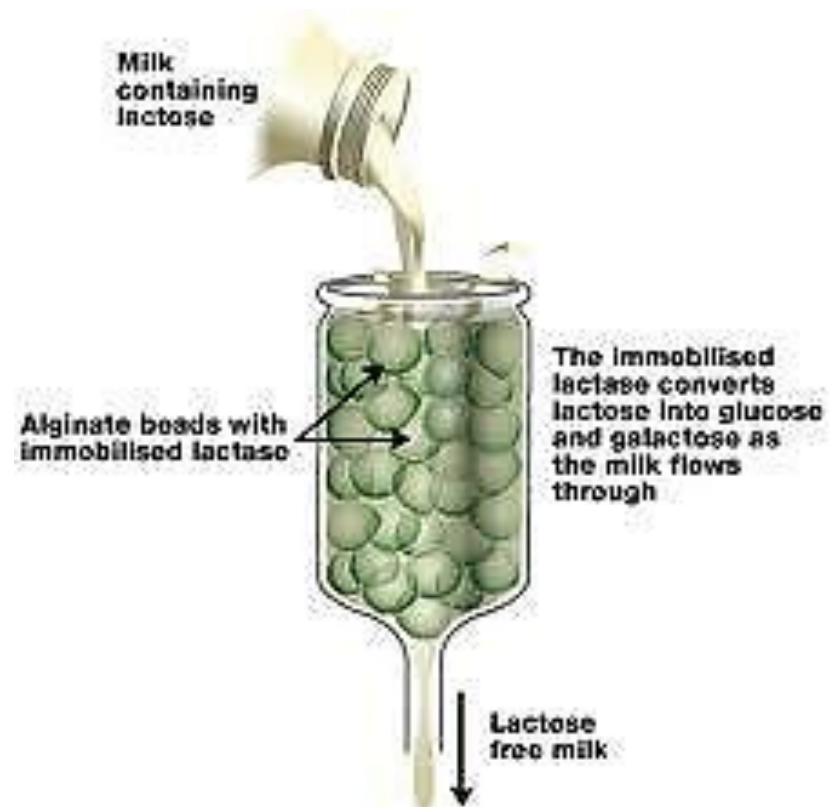
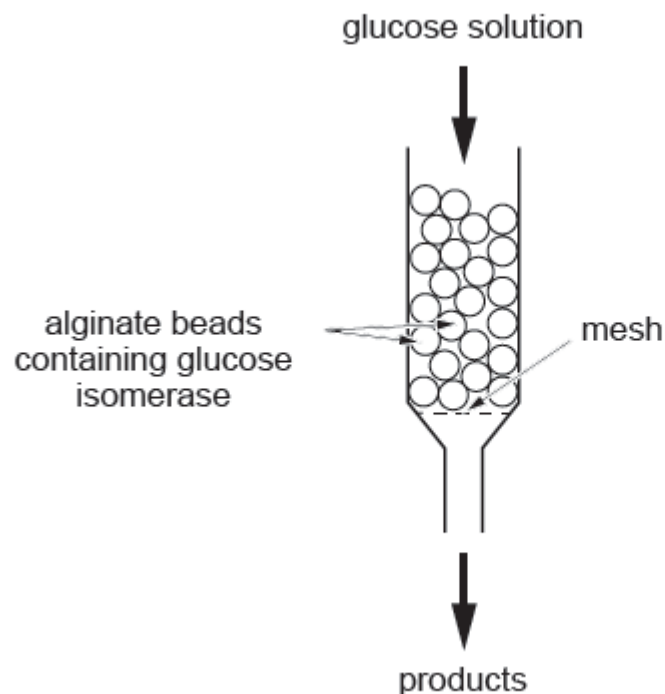
- Mix enzymes with **sodium alginate**
- Add drop by drop into **calcium chloride**
- Pack them into a **column**



Immobilised Enzymes

4. Run substrates through the column
5. Collect products from bottom

You can run the products through the column again and again to increase % yield.



Why Use Immobilised Enzymes?

- Can easily **reuse** enzymes
- Can easily recover enzymes
- Longer shelf-life of enzyme
- **Less purification / downstream processing** needed
 - Bcs it keeps product **enzyme-free** so no effect on product quality
- Allow **continuous production**
 - Can obtain more product per unit time
- Reduces end product inhibition
- Enzymes more tolerant of **pH** changes
- **Thermostable** – less likely to denature at high temp

Tolerance to pH and temperature

- Alginate **protects enzyme**

→ As enzyme is less exposed to solution

→ **Less H^+ or OH^- penetrate the alginate beads**

→ Shape of **active site** of immobilised enzyme is less disrupted

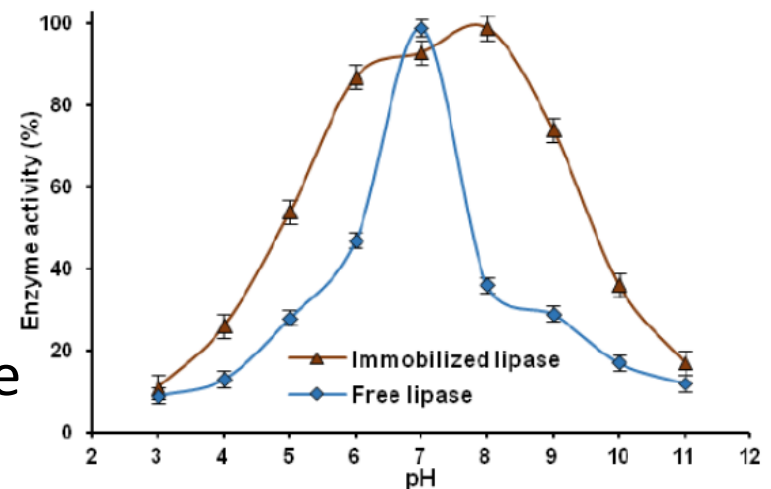
- 3D structure of enzyme is **stabilized**

→ **H bonds vibrate less** at high temp

→ Fewer bonds within immobilised enzyme break

→ **Active site** less likely to change shape

→ Less denaturation



Chapter Outline

Part 1: Enzyme Structure and Mode of Action

- Enzyme Structure
- Lock and Key Hypothesis vs Induced Fit hypothesis
- Mode of Action

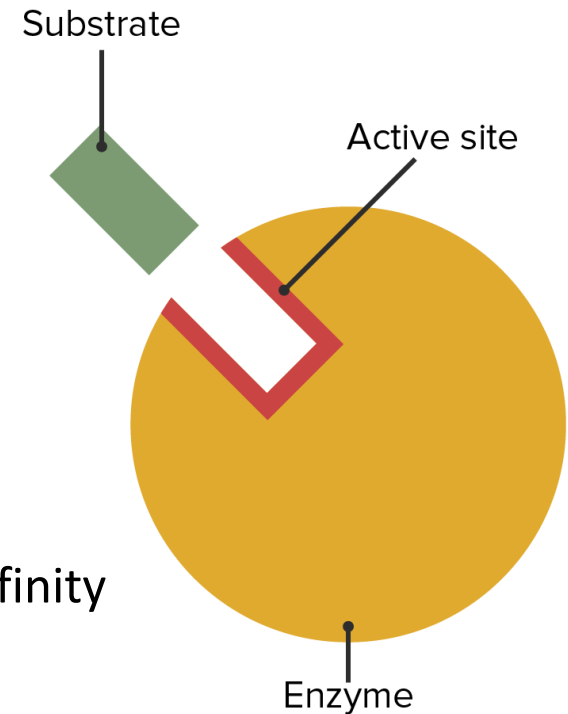
Part 2: Mechanism of Enzymatic Reaction

- Measuring the rate of reactions
- Time course graph

Part 3: Factors Affecting Enzymatic Reaction

- Substrate concentration
 - Maximum rate of reaction ($V_{\max}$)
 - Michaelis-Menten constant (K_m) – a measure of affinity
- Enzyme concentration
- Temperature
- pH
- Competitive vs Non-competitive Inhibition

Part 4: Immobilised enzymes



Useful Videos

- Explaining enzyme inhibitors and the graphs

https://www.youtube.com/watch?v=p08Z_fyErWg



Links below cover things you SHOULD NOT write in exams
but maybe helpful for your understanding / for your entertainment!

- How to speed up chemical reactions (and get a date)

<https://www.youtube.com/watch?v=OttRV5ykP7A>

- How do you explain Michaelis Menten to a kid?
(this is more detailed than needed)

<https://www.youtube.com/watch?v=6KCRfY5mwkU>